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(54) Title: AGENT AND METHOD FOR MODULATION OF CELL MIGRATION

(57) Abstract

A GON-1 migration protein in *C. elegans* and a *gon-1* gene encoding same are disclosed. The protein, termed GON-1, shows structural similarity to a protein produced by an up-regulated RNA in an advanced tumor cell. Although the tumor cell protein has not previously been identified as having any role in cell migration, it is disclosed herein that the related GON-1 protein is required for cell migration and is involved in shaping tissues or organs. It is deduced that the protein is also a target for modulators of cell migration and tissue shaping.

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AGENT AND METHOD FOR MODULATION OF CELL MIGRATION

BACKGROUND OF THE INVENTION

Cell migration, particularly migration of cancerous cells and nerve cells, is not well understood, nor are the factors that affect cell migration and tissue shaping *in vivo*. There is a need in the art to identify and exploit such factors, including but not limited to those involved in normal or abnormal organogenesis. The art also lacks efficient systems for evaluating therapeutic modulators of such functions *in vivo* and lacks diagnostic methods for assessing the ability of a cell or cell mass to migrate *in vivo*.

Organogenesis processes in vertebrates proceed in a manner similar to those observed in the common laboratory nematode *C. elegans*. As such, the generation of *C. elegans* gonadal structures can serve as a simple system for investigating developmental morphogenetic processes shared by higher and lower organisms.

In one common morphogenetic process, a tissue bud extends to form an elongate tube with a proximal to distal axis. An emerging theme in bud extension is the presence of specialized regulatory cells at the bud tip that govern elongation. In vertebrate development, this process is

seen in extension of the limb (Johnson and Tabin, 1997; Martin, 1998), ureter (Vainio and Muller, 1997), and lung branches (Hogan, 1998). In the *C. elegans* gonad, long "arms" develop by elongation of buds originating from a gonadal primordium. Each gonadal arm possesses a single "leader cell" that serves this regulatory role (Kimble and White, 1981). The biology of distal tip cell migration during gonadogenesis is known to one skilled in the art of *C. elegans* developmental biology. Indeed, the *C. elegans* gonadal leader cells are among the best defined cells that regulate bud elongation, and therefore serve as a paradigm for investigating this common morphogenetic process.

A second common morphogenetic process of organogenesis is the formation of a complex, differentiated epithelial tube. Formation of a complex epithelial tube can involve an initial condensation of mesenchymal cells, followed by epithelialization, lumen formation, and differentiation into modular units. Vertebrate examples include the kidney tubules (Vainio and Muller, 1997) and heart tube (Fishman and Olson, 1997). Similarly, during *C. elegans* gonadogenesis, cells coalesce to form a compact larval structure called the somatic gonadal primordium (SGP). Following formation of this primordium, cell division and differentiation are accompanied by epithelialization and lumen formation to form a complex tube composed of distinct modular units: the uterus, spermathecae and sheaths in hermaphrodites, and the seminal vesicle and *vas deferens* in males (Kimble and Hirsh, 1979).

Previous studies have identified several genes in *C. elegans* that influence gonadal morphogenesis. One group of such genes includes *unc-5*, *unc-6*, and *unc-40*, which control the direction of leader cell migration (Hedgecock et al, 1990). Normally, leader cells migrate in one direction, then move dorsally, and finally move in the opposite direction to generate a reflexed gonadal arm. In the absence of *unc-5*, *unc-6*, or *unc-40*, the leader cells fail to turn dorsally. Another gene, *ced-5*, causes the

leader cell to makes extra turns or stop prematurely (Wu and Horvitz, 1998). Therefore, in these mutants, the leader cells migrate, but do not navigate correctly, which results in a failure of the gonadal arms to acquire their normal U-shape. In addition to these genes, others are required for specification of cell fates and also influence morphogenesis (*lin-12:* Greenwald et al., 1983, Newman et al., 1995; *lin-17:* Sternberg and Horvitz, 1988; *lag-2:* Lambie and Kimble, 1991; *ceh-18:* Greenstein et al., 1994, Rose et al., 1997; *lin-26:* den Boer et al., 1998).

A known *C. elegans* genetic locus, *gon-1*, defined by one or more mutants, is essential for extension of gonadal germline arms, but is not responsible for signaling the germline to proliferate. In *C. elegans* hermaphrodites, GON-1 is required for migration of two distal tip cells to produce two elongated tubes, whereas in males, *gon-1* activity is required for migration of a single linker cell to produce a single elongated tube. In *gon-1* mutant hermaphrodites, the leader cells are born normally in the somatic gonadal cell lineage and function normally to promote germline proliferation, but they fail to migrate and do not support arm extension. Similarly in males, the leader cell does not move and no arm extension occurs. The *gon-1* locus has not heretofore been mapped with particularity to a nucleic acid coding sequence.

Clarification of the genetic basis for *C. elegans gon-1* activity would permit one to apply molecular tools to the study of cell migration in a convenient system. It would be particularly advantageous to find that the *gon-1* locus encodes a protein having structural relationship to proteins of species that are not readily studied in the laboratory, since one would be able to evaluate those proteins in the convenient *C. elegans* system. Such a system would also provide a means for evaluating agents that can modulate the activity of such genes and proteins and would both facilitate understanding the factors involved in cell migration.

BRIEF SUMMARY OF THE INVENTION

In one aspect, the invention can be an isolated polynucleotide coding sequence that encodes a protein the includes both a metalloprotease domain and at least one thrombospodin type 1 domain, where the protein can direct either cell migration or tissue shaping in an analytical system in a target organism as disclosed herein. In another aspect, the invention can also be a variant of the isolated polynucleotide coding sequence that encodes a protein that shares at least 20%, more preferably 50%, still more preferably 70% and most preferably 80% amino acid sequence identity (using GCG Pileup program) with any of the foregoing in the metalloprotease and thrombospondin type 1 domains while also comprising the amino acids of those domains known to those skilled in the art to be required for protein activity. A suitable variant polynucleotide can hybridize under stringent hybridization conditions known to those skilled in the art to a polynucleotide sequence that encodes a protein that can direct cell migration or tissue shaping in the target organism. In one embodiment, a variant polynucleotide can hybridize under stringent hybridization conditions to a *C. elegans gon-1* coding sequence. The variant polynucleotide sequence can be a polynucleotide obtained from an organism or can be a mutated version of any polynucleotide sequence noted above. The variant polynucleotide can encode a protein that is identical or altered relative to the wild-type *C. elegans* GON-1 protein. The encoded protein can have enhanced or reduced activity *in vivo* relative to GON-1.

In a related aspect, a polynucleotide coding sequence that encodes a protein having structural and functional similarity with a wild-type or altered migration or shaping protein can also be substituted, in whole or in part, with structurally related or unrelated sequences to encode a heterologous protein or a chimeric protein in the disclosed system, as detailed below.

Applicants herein disclose that the *Caenorhabditis elegans gon-1* activity is encoded by a polynucleotide coding sequence (*gon-1*; SEQ ID NO:1) that encodes an essential protein (GON-1; SEQ ID NO:2) that directs migration of a growing gonadal tube through surrounding basement membranes during gonadogenesis in the nematode and also controls gonadal shape and organ localization.

The migration directing ability and tissue shaping ability are separable and depend upon whether the *gon-1* coding sequence is expressed in distal tip cells or in muscle cells, respectively. In wild-type *C. elegans*, a gonad of normal shape is produced when *gon-1* is expressed in both cell types. Accordingly, one aspect of the invention can also a method for shaping a tissue by selectively expressing a protein associated with both tissue elongation and tissue expansion. GON-1 shares significant amino acid identity with proteins that have been noted in other species.

In a related aspect, the invention can be an isolated and substantially purified preparation of a GON-1 protein, an altered GON-1 protein, a heterologous protein, a chimeric protein, or a variant thereof (referred to herein as "an MPT protein", for reasons discussed below), which can be a target for *in vivo* screening of putative therapeutic modulators, or can be assayed in a diagnostic method for assessing the ability of a cell or cell mass to migrate *in vivo*, or can be exploited as a therapeutic agent to modulate (increase or decrease) *in vivo* cell migration.

One skilled in the art will appreciate that the nucleotide coding sequences and encoded amino acid sequences that fall within the scope of the invention are also subject to natural variation or intentional manipulation (e.g., changes in the nucleotide or amino acid sequence) in ways that do not affect the ability to function as described herein. One skilled in that art also understands that the applicants cannot provide a complete list of nucleotide coding sequences and amino acid

sequences that can function in the methods of the invention. However, in view of the high level of understanding in the art about the amino acids required for activity of proteins that comprise a metalloprotease domain and proteins that comprise a thrombospondin domain, applicants maintain that a skilled artisan can readily determine whether a protein contains both domains. Stöcker, W. et al., "The metzincins - Topological and sequential relations between the atacins, adamalysins, serralysins, and matrixings (collagenases) define a superfamily of zinc-peptidases," Protein Science 4:823-840 (1995), Rawlings, N.D. and A.J. Barrett, "Evolutionary families of metallopeptidases, Methods in Enzymology 248:183-228 (1995), and Adams, J.C. et al., The Thrombospondin Gene Family, R.G. Landes Company, Austin, TX (1995), all incorporated herein by reference in their entirety, provide sufficient guidance to permit those in the art to establish whether a protein comprises both a metalloprotease and a thrombospondin domain.

The invention is further summarized in that an antibody can be produced against characteristic epitopes of any of the foregoing proteins using standard methods. The antibody can be used both diagnostically to ascertain the presence of an MPT protein, or therapeutically to interfere with activity of the MPT protein.

The present invention is also summarized in that an animal that contains a *gon-1* allele (or homolog or variant thereof) is a convenient screening tool for finding modulators of cell migration. The present invention is thus further summarized in that a method for identifying modulators of the disclosed MPT proteins includes the steps of treating a target organism having a cell that can migrate or be shaped when under control of an MPT protein with at least one potential modulator of migration or shaping and observing in the treated target organism a change in migration or shaping of the cell or tissue attributable to the presence of a modulator. In a

preferred embodiment, the cell is a developing gonadal cell in *C. elegans*, although other cells or organs may be similarly regulated by MPT proteins in other organisms.

The ability of the MPT protein to direct a cell or tissue under its influence to migrate or be shaped can be modulated (increased or decreased) in a variety of ways, such as by altering the migration protein's primary, secondary, or tertiary structure, by altering the location or amount of the protein in an organism, by altering the transcriptional or translational regulation of the gene that encodes the protein, or by providing the organism with an agonist or antagonist molecule in an amount sufficient to interact with the MPT protein so as to increase or decrease the ability of the protein to direct migration or shaping.

In a related method, one can also identify nucleic acid sequences required or desired for migration or shaping of such a cell, by treating a target organism with an agent that affects the polynucleotide sequences of the target organism that encode the MPT protein or that participate in regulating expression of the MPT protein, and then identifying sequences affected by the treatment. The sequences identified in the method can be either complete or partial coding sequences or can be regulatory sequences.

It is an object of the present invention to identify a protein and nucleotide sequence encoding same that directs migration or shaping of a cell or tissue.

It is another object of the present invention to provide a method for modulating cell migration or shaping.

It is yet another object of the present invention to provide a system and method for screening putative modulators of migration or shaping of cells or tissues.

It is an advantage of the present invention that agents having a putative effect upon migration or shaping can be screened in a convenient model system rather than in a vertebrate organism.

Other objects, features and advantages of present

invention will become apparent upon consideration of the following detailed description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Fig. 1A depicts a schematic map of the *gon-1* locus in *C. elegans* from which the gene was cloned and shows the exon-intron structure of *gon-1*.

Fig. 1B shows a schematic map of *C. elegans* GON-1, the location of five protein-truncating stop mutants in GON-1 and a comparison to the protein structures of the murine ADAMTS-1 protein, and the bovine procollagen-I N-proteinase (PN1P) protein. From left to right, GON-1 includes a prodomain, a metalloprotease domain, a first cysteine rich region, a thrombospondin type I motif, a second cysteine rich region, and a plurality of thrombospondin type I-like motifs. The five mutants are identified as *q518 (aa591 TGG->TGA), e2551 (aa1069 TGG->TAG), e2547 (aa1229 TGG->TGA), q18 (aa1234 TGG->TAG)* W->stop, and e1254 (aa1345 CGA->TGA) R->stop).

Fig. 1C compares the *C. elegans* GON-1 amino acid sequence to sequences of the ADAMTS-1 and PN1P proteins. In the metalloprotease domain, amino acids important for enzymatic activity are marked by an asterisk (*). Three conserved histidines (GON-1, aa 424, 428, 434) bind a catalytically essential Zn^{+2} ion in well characterized metalloproteases, while a glutamic acid residue (GON-1, aa 425) is thought to be directly involved in cleavage (Stöcker et al, 1995). In addition, two conserved glycines and a downstream methionine seem to be important for structure of the active site. GON-1 bears one of the glycines (aa 427) and the methionine (aa 454), but the second glycine is changed to serine in GON-1 (aa431). In the canonical TSPt1 domain, amino acids conserved in vertebrate TSP type-1rpeats are shown by a plus (+). The mutation, *gon-1(q518),* is marked by an inverted triangle

(V). For the TSPt1-like repeats, only 2 of the 17 are shown. The consensus sequence for these repeats is: $W-X_{4-5}-W-X_2-$ $CS-X_2-CG-X_{4-5}-X-G-X_3-R-X_3-C-X_{4-27}C-X_{8-12}-C-X_{3-4}-C$. Because only the first two TSPt1-like motifs are shown, the other mutations are not indicated in this figure.

Fig. 2A depicts normal morphogenesis of the *C. elegans* hermaphrodite gonad.

Fig. 2B shows that arm extension does not occur in *gon-1* mutants and that the gonad develops as a disorganized mass of somatic and germline tissues. Similarly, in males, the *gon-1* mutant gonad is severely disorganized and does not acquire its normal shape.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The existence of a protein in *C. elegans* required for cell migration or shaping has not heretofore been known, nor has any function been previously ascribed to a protein encoded by the designated sequence. The inventors have determined that a functional GON-1 protein is required for migration of the regulatory cells that lead the developing gonad organ during its migration. GON-1 is also involved in shaping tissues such as gonads. By appreciating the role of GON-1 (and the *gon-1* gene) and its relationship to a related gene that is upregulated in a metastatic tumor cell, the inventors have identified a gene and protein believed to be fundamental in the process of normal and abnormal cell migration and tissue shaping. The gene and protein, and related genes and proteins, can be utilized in the methods of the invention as described herein. References herein to influencing cell migration are also intended to encompass shaping of tissues or organs. Likewise, references to a migration protein encompass proteins of the same class that can also be used in methods for shaping tissues or organs.

Generally speaking, the methods of the present invention permit one to identify agents that modulate cell migration or tissue shaping *in vivo* or *in vitro*. One can

treat target organisms with panels of polynucleotides, proteins, sugars, lipids, organic molecules, other chemicals, synthetic or natural pharmaceutical agents or other agents to determine whether any agent affects activity of an MST protein. This list is necessarily incomplete, since one cannot predict in advance which agents will be effective. However, applicants have enabled a system for screening panels of putative agents, in accord with the common practices of pharmaceutical companies that typically screen thousands of compounds against a test system in an effort to reveal preferred agents. Candidate agents likely to modulate MPT proteins in the disclosed system include tissue inhibitors of metalloproteases and pharmaceutical metalloprotease inhibitors or enhancers such as those from British Biotech. Inhibitors or enhancers of thrombospondin activity are also good candidate agents.

Agents so identified can be used therapeutically to enhance or inhibit cell migration or to influence tissue shape. Agents having an adverse or inhibiting or knock-out effect upon activity of a migration protein can also be used in a method for biocontrol of animals that employ the migration protein in gonadal development, where the method includes the step of exposing a developing animal to an amount of the agent effective to prevent gonadal development such that the animals are rendered sterile. While this biocontrol method is particularly envisioned for use in nematodes, it may be applicable to other animals as well, since genes related structurally and functionally to *gon-1* are known to exist in animals as diverse as nematodes, cattle and humans.

Using the invention one can also identify polynucleotide sequences including coding and regulatory sequences that affect activity of a migration protein. For example, null or so-called reduced activity mutants can be mutagenized and assayed for activity-restoring, activity-inhibiting or activity-enhancing changes. By extension, one can perform comparable screens *ad infinitum* on

sequences identified in this manner, to obtain still more sequences that have an indirect effect on migration activity. After identifying such sequences in a target organism, one can obtain homologous polynucleotides from other organisms by screening nucleic acid libraries under stringent hybridization conditions in a manner known to those skilled in the art.

A method for evaluating putative modulators of cell migration preferably employs a nematode as a target organism. The methods may be advantageously practiced using a nematode that comprises a migration protein as described herein, or a mutant nematode that either lacks a migration protein or contains a migration protein having reduced activity. The protein can be encoded by wild-type *C. elegans gon-1* (disclosed herein), by a mutant that confers upon the nematode an enhanced or reduced sensitivity to modulators, by a transgene from another organism, in whole or in part, or by a variant of any of the foregoing. Nematodes are desirable target organisms, in general, because they are easy to grow and maintain, and easy to assay, particularly because they are transparent.

Nematodes are also particularly desired because the powerful techniques of reverse genetics can be employed. One can also target specific *C. elegans* sequences for mutation or RNA-mediated interference (a technique used to transiently knock genes out by RNA injection) to identify nucleic acid and protein sequences that have a direct inhibitory or enhancing effect on *gon-1* activity.

With the identification of the *gon-1* gene and GON-1 protein in *C. elegans* and the discovery of homologous genes in other species, the functions of migration proteins can be analyzed *in vivo* during organogenesis using the full force of molecular genetics available in that system. Such functions can include, but may not be limited to cell migration, basement membrane remodeling, and tubular organ formation.

Although the system is exemplified in *C. elegans*, a

free-living (i.e., non-parasitic) nematode, those skilled in the art can develop similar systems operating on the same principles without undue experimentation in other convenient organisms, including other nematodes including, without limitation, *C. briggsae*, or in, for example, *Drosophila*, or other organisms conveniently studied in the laboratory. To do so, one would only need to identify the homolog of *gon-1* in such an organism, using standard molecular biological methods and then screen for related genes, proteins and other factors as described herein. One could also use such systems in other animals to study transgenes in ways comparable to those described herein. Those skilled in the art can produce transgenic animals of many species without undue experimentation.

In the method, a putative modulator is provided to the target organism, for example, by adding it to the growth media, by injecting it into the organism or by gene transformation technology. The effects of said modulator can be assessed either by screening for changes in cell migration or by genetic selection for fertile animals. The assessment methods are known to those skilled in the art. *Caenorhabditis elegans*: Modern Biological Analysis of an Organism, Methods in Cell Biology, volume 48, Epstein, H. F. and D. C. Shakes, eds., Academic Press (1995), incorporated herein by reference in its entirety, describes suitable methods and conditions for growing and monitoring *C. elegans*.

C. elegans GON-1 is characterized by a multi-domain structure that includes several known motifs. GON-1 protein is a secreted metalloproteinase that lacks a transmembrane domain and possesses a predicted metalloprotease domain between amino acids 269-456. The metalloprotease enzymatic activity is essential for GON-1 function; proteins that might be cleaved by this metalloproteinase include components of the basement membrane and other proteins that modulate migration. The metalloprotease domain shares sequence similarity with other metalloproteinase enzymes.

In addition to its metalloprotease domain, GON-1 possesses a series of consecutive motifs that are related to, but variants of, the thrombospondin type 1 (TSPt1) repeats (Fig. 1B,C). The most N-terminal TSPt1 repeat bears the hallmarks of this type of motif in vertebrate thrombospondins (15/16 of the consensus amino acids, + in Fig. 1C) (Adams et al., 1995), whereas the remaining 17 repeats are less similar and define a TSPt1-like variant. Proteins that might interact with this domain include proteins that modulate migration, including but not limited to components of the basement membrane.

GON-1 is similar to members of the reprolysin subfamily (Rawlings, N.D. and A.J. Barrett, "Evolutionary families of metallopeptidases, Methods in Enzymology 248:183-228 (1995), incorporated herein by reference in its entirety). At the N-terminal border of the metalloprotease domain, there is a potential furin cleavage site (Fig. 1C) (Pei and Weiss, 1995; Pei and Weiss, 1996). GON-1 and the reprolysins share a common zinc binding active site with the larger metzincin superfamily (Stöcker et al., 1995). Amino acid conservation within the active site together with the known crystal structure of several superfamily members reveals those amino acids essential for enzymatic activity (marked by asterisks in Fig. 1c) (ibid). GON-1 has all amino acids implicated in catalysis and all but one implicated in structure of the active site.

Wild-type *C. elegans* GON-1 (SEQ ID NO:2) is suitable for use in the methods of the present invention, although a skilled artisan can replace the *C. elegans gon-1* coding sequence with a sequence that encodes all or part of a homologous protein, using the standard tools available to a molecular biologist. This mixing and matching can increase or decrease the activity of the encoded chimeric protein. As described elsewhere herein, it can be desirable to provide a system having reduced or enhanced migration activity, or even no migration activity, depending upon whether one is evaluating agents that enhance or inhibit

migration. Increased gene activity is characterized either by increased gonadal arm extension, increased compactness of gonadal tissue, or fertility. Decreased gene activity is assayed either by decreased gonadal arm extension, decreased compactness of gonadal tissue or sterility. Certain specific activity-reducing mutations in *gon-1* are described in the Examples.

Sequences with related structures have already been isolated from vertebrate organisms, but no related invertebrate sequence is known to the inventors. Still other related metalloprotease proteins (and polynucleotide sequences encoding same) will be isolated from vertebrate and invertebrate organisms. While the *C. elegans gon-1* protein includes 17 thrombospondin domains, the bovine and murine homologs include only 2 such domains. Other known members of the family also have one canonical TSPt1 repeat, can contain at least one TSPt1-like variant repeat, and contain two conserved cysteine rich regions. Based on this conserved architecture, we suggest the name MPT (for MetalloProtease with TSP1 repeats) for the family.

While the *in vivo* functions of these proteins may differ from that of *C. elegans* GON-1, these proteins are expected to function in place of GON-1 in whole or in part in the disclosed methods. All such homologs from other vertebrate and invertebrate organisms (and the polynucleotide sequences that encode such homologs), variants thereof, and chimerics that incorporate portions thereof, whether obtained naturally or induced in the laboratory using the tools available to a molecular biologist, are considered to be useful in the present invention. In particular, functional domains, such as the metalloprotease domain, can be swapped into corresponding domains in *gon-1*.

The amino acid sequences of GON-1, ADAMTS-1 and bovine PN1P are compared in Fig. 1C. The additional thrombospondin domains of GON-1 not found in ADAMTS-1 or PN1P are not shown in Fig. 1C. Those portions of GON-1

that have no obvious relationship to known motifs are conserved among the family of GON-1 homologs. The GON-1 protein shows significant sequence similarity to the bovine procollagen-1 N-proteinase (P1NP), to the murine ADAMTS-1 protein, and to a pair of human aggrecan-degrading metalloprotease-encoding sequences described in International Patent Application Number PCT/US98/15438, published on February 4, 1999 as International Publication No. WO 99/05291, incorporated herein by reference in its entirety. Another human homolog which has significant identity to the bovine P1NP has Genbank accession number d1021662.

Bovine P1NP can proteolyze the N-terminal propeptide from collagen I (Colige et al., 1995, Colige et al., 1997). Metalloprotease activity is required for GON-1 function and suggest that, like P1NP, it may cleave components of the extracellular matrix. Murine adamts-1 expression correlates with tumor cell progression (Kuno et al., 1997). The murine ADAMTS-1 protein is found in an advanced cachexogenic murine tumor cell. Human aggrecanase has been associated with arthritis in humans. Given the role of GON-1 in regulating cell migration of the C. elegans leader cell, we suggest that MPT proteins may be involved more generally in cell migrations that must pass through extracellular matrix and that, in cancerous tissues, loss of MPT regulation may promote metastasis. The percent identity of the identified domains of *C. elegans* GON-1 with the bovine and murine proteins is shown in Fig. 1B.

Changes can be made in any of the foregoing at the nucleic acid level in a manner known to those skilled in the art, by, for example, removing a section of the coding sequence, interrupting the coding sequence with an additional sequence, rearranging at least one section of the gene, or by providing in the sequence other changes that can include but are not limited to point mutations that either truncate the protein or disable an active site in the protein encoded by the altered polynucleotide.

Changes can also be made by altering the transcription or translation of the gene that encodes the migration protein by altering in a manner known to the art the upstream and/or downstream regulatory sequences that the surround the gene. Likewise the translation-regulating elements of an mRNA encoding the migration protein can also be altered to affect the stability or location of the mRNA. An antisense RNA can also interfere with translation of the migration protein.

At the protein level, one skilled in the art can modulate the activity of the migration protein either by modifying the protein encoded by the gene as noted above or by directing the protein to be modified in vivo, for example, by providing in the protein appropriate signal or signals for cleavage or degradation by other cellular factors. Alternatively, the protein can be targeted with an activity-modulating factor such as a protein, a peptide, or an organic or inorganic co-factor. Any of these factors can, for example, occupy or obstruct an active site of the protein which is required for activity. Likewise, if the activity of the protein is natively regulated by an endogenous co-factor, an effect can be achieved by modulating the availability of the native co-factor.

One skilled in art is familiar with the techniques associated with the aforementioned alterations, including the production of any construct necessary to effect such changes. One skilled in the art also understands that changes in the primary amino acid sequence (including, e.g., substitutions, deletions, additions, inversions) may or may not alter the activity of a protein, depending upon the position and the extent of the change.

For purposes of this application a migration protein is considered active if it causes a cell that comprises the protein, or a cell that is under the influence of the protein, to migrate to any appreciable extent. A cell is "under the influence of the protein" if the cell migrates in the presence of the protein, even if the cell does not

contain the protein. *In vivo*, the cell from which the protein is secreted and its site of action remain unknown.

Non-native transgene sequences containing non-native sequences homologous to all or part of *C. elegans gon-1* can be introduced into *C. elegans* on an expressible genetic construct that contains a promoter that drives expression in a tissue that allows easy assay so that the effect or effects of those sequences on migration and other functions can be evaluated in the system. Methods for generating and selecting transgenic nematodes are well-known in the art. Transgenic animals can rescue null mutants or can suppress or enhance the activity in the reduced-activity mutants. A preferred example of a transgene sequence is a human *gon-1* homolog sequence, although any of homolog can be used. Some constructs may contain all or part of the *gon-1* coding sequences. The transgene should be appropriately expressed near the cells to be controlled by the migration protein. In *C. elegans*, the *gon-1* promoter, active in leader cells and in muscle cells, is suitable. Other promoters that can be used in *C. elegans* include the *lag-2* promoter, which drives expression in the hermaphrodite distal tip cells, and the *unc-54* promoter which drives expression in body wall muscle.

One can assay for effects of treatment with a potential modulating agent on cell migration and gonadal tube extension by comparing migration after treatment to the cell migration in either a wild-type organism or to that in an untreated, previously characterized mutant. Before treatment in the methods, if the migration protein is expressed in leader cells at wild-type levels, directed elongation of gonadal arms along a proximal-distal axis is observed. If the migration protein is expressed in muscle, on the other hand, one observes more dispersed activity, which may be important for expansion as the gonad along the dorsal-ventral and left-right axes. If a migration protein having a level of activity comparable to that of the wild type protein is expressed from a polynucleotide sequence

under control of the native *gon-1* promoter, of course, normal gonadal development is observed, as is shown in Fig. 2A. Fig. 2B shows that arm extension does not occur in *gon-1* mutants and that the gonad develops as a disorganized mass of somatic and germline tissues. Similarly, in males, the *gon-1* mutant gonad is severely disorganized and does not acquire its normal shape. Both wild-type activity and the mutant phenotype can be modified by treatment according to the methods. One can also direct the shape of a tissue or organ by introducing a transgene coding sequence under control of a promoter selected to express the transgene coding sequence in a desired tissue or cell type.

One can also assess whether a cell has the potential for migration by analyzing for example, the level of the migration protein in the cell, or the level at which the RNA encoding the migration protein is present. A diagnostic assay for the presence of active site residues in the protein can also be devised. Likewise, the presence or absence of a DNA sequence encoding an essential aspect of the protein can also be used in a diagnostic manner to assess the likelihood of cell migration.

Our finding that GON-1 is tightly regulated to achieve arm extension during gonadogenesis in C. elegans suggests that similar activities may play similar roles in the morphogenesis of organs throughout the animal kingdom. Previous *in vitro* experiments support this notion. For example, antibodies recognizing matrix metalloprotease 9 (MM9) can block branching of the ureter bud during kidney development (Lelongt et al., 1997), and inhibitors of MMPs block the invasion of endothelium cells into a fibrin matrix in assays for angiogenesis (Hiraoka et al., 1998). Based on these observations and our analysis of GON-1, we suggest that the MPT metalloproteases are critical modulators of organogenesis.

Whether the target organism contains a wild-type *C. elegans gon-1* gene, a mutant *gon-1* gene or a transgene substituted in place of *gon-1*, in whole or in part, the

system is readily used to identify other genes, proteins, drugs, chemicals or other factors that either enhance or antagonize activity.

In a method for increasing the migration of the cell, the native protein or related protein or a genetic construct encoding same can be administered to, or caused to be expressed at a high level in, the target cell. Alternatively, an enhancing factor can be provided inside or outside the target cell, as appropriate. Where it is desired to decrease migration of a targeted cell, as in the case of a tumor cell, an inhibiting factor can be added into, or the vicinity of, the targeted cell. The vicinity of the cell is defined as sufficiently close to the targeted cell so as to effect a desired change in the cell migration. If the migration protein is secreted from the cell in which it is produced, the activity of the protein can further be modulated either by preventing secretion of the protein or by interfering with the protein activity outside the cell. If the protein acts outside the target cell, the protein, an active portion thereof, or a modulating factor can be administered to the vicinity in an amount effective to modulate cell migration.

The reproductive sterility that can result from inhibited migration of developing gonadal cells under the control of an migration protein that is inactive or has reduced activity can be further exploited, for example, in a method for controlling reproduction of an organism that relies upon a migration protein during gonadogenesis. An organism for which such control would be appropriate would include *C. elegans* and other nematodes or parasites, and could include other invertebrates, as well as vertebrate species including, for example, avian, amphibian, reptilian and mammalian species.

With an appreciation for the migration proteins of the invention, normal and abnormal cell migration attributable to activity of a migration protein can be therapeutically increased or decreased. The mechanisms by which the gene

and protein are regulated can be determined by one skilled in the art and can be advantageously exploited to modulate expression of the migration protein at either the nucleic acid or protein levels.

EXAMPLES

To gain molecular insight into *gon-1* function, we cloned the gene by a combination of fine genetic mapping, mutant rescue and RNA-mediated interference. Mutations in the *gon-1* gene were finely mapped by genetic crosses with respect to markers that had already been placed on the physical map. Cosmids in the region were next tested for mutant rescue of the *gon-1* mutations. The genomic *C. elegans* sequence that includes the coding sequence of the *gon-1* gene in a plurality of exons is found on cosmids F25H8 (Accession # 69360) and T13H10 (Accession #69361); T13H10 bears most of *gon-1* and rescued the *gon-1* phenotype. The predicted open reading frames on this cosmid were tested by RNA-mediated interference to identify the transcript corresponding to *gon-1* activity. The identification of this transcript as *gon*-1 was then confirmed by subcloning and mutant rescue by a smaller region of the cosmid that contained that transcript, by RNA-mediated interference, and by identifying *gon-1* mutations in the coding region of this transcript. The positions in the migration protein that correspond to the identified mutations are indicated in Fig. 1B. We confirmed identification of F25H8.3 as *gon-1* by identifying molecular lesions for a plurality of *gon-1* alleles.

Mutants were obtained as described (Brenner, S. "The Genetics of *Caenohrabditis elegans*, Genetics 77:71-94 (1974), incorporated herein by reference. Each contained an allele of *gon-1* that maps to chromosome IV between *unc-24* and *dpy-20*, all are recessive, and all are fully penetrant for sterility. Five alleles, *e1254*, *e2547*, *q18*, *q517*, and *q518*, fail to complement the sixth allele, *e2551*, and, therefore, the mutations define a single gene. Three-factor mapping places *gon-1(e2551)* 0.08 map units to

the right of *elt-1* and 0.12 map units to the left of *unc-43* at position 4.44. Specifically, among Unc-43 non-Elt-1 recombinants isolated from *gon-1/ elt-1 unc-43* mothers, 8/13 carried the *gon-1* mutation.

To compare allelic strengths, we examined the penetrance of arm extension defects in homozygotes for each allele. In *gon-1(q518)* homozygotes, no arm extension was observed at 15°, 20° or 25°C. However, in homozygotes for the other *gon-1* alleles, some arms extended at least partially. By this measure, the *gon-1* alleles can be placed in an allelic series: *q518* < *e2547* ≈ *q18* < *e1254* ≈ *q517* < *e2551*. Interestingly, the weaker *gon-1* alleles have a more severe defect at lower temperature, which may reflect a cold sensitivity of GON-1 function, or of the process of arm extension itself.

The strongest loss-of-function allele is *gon-1(q518)* which is a nonsense mutation that resides in the canonical TSP1 motif; the other mutations are located in the TSP1t1-like repeats. *gon-1*(q518), the nonsense mutant located closest to the N-terminus, has the most severe effect on cell migration; nonsense mutants located closer to the C-terminus than q518 are partially defective for migration. Because the mutant phenotype for *gon-1(q518)* homozygotes is identical to that of *gon-1(q518)* hemizygotes and because *gon-1(q518)* bears a nonsense mutation predicted to remove the bulk of the GON-1 protein, this allele is likely to be a molecular null. Therefore, *gon-1(q518)* was used for analyzing the roles of *gon-1* in gonadal morphogenesis and is referred to as *gon-1(0)*.

Normally, the gonad is a tubular structure with specialized regions. By contrast, in *gon-1* mutants, the adult gonadal tissues exist as a disorganized mass with little or no tubular morphology. Specifically, neither arms nor somatic gonadal structures (e.g. uterus, spermatheca) are observed. In all cases, however, the gonads are rendered infertile by these mutations.

In *C. elegans*, mRNAs containing premature stop codons

are normally degraded by the *smg* system, but those mRNAs are stabilized in a *smg* mutant background (Anderson and Kimble, 1997). Therefore, the remaining activity of truncated GON-1 proteins should be evident in *smg-1; gon-1* double mutants. We found that *gon-1(q518)* was not suppressed in a *smg* background, whereas all four mutations in the TSP1-like repeats were suppressed. Therefore, while the GON-1(q518) mutant protein that possesses the metalloprotease domain but lacks the *bona fide* TSPt1 motif (as well as the rest of the protein C-terminally), is not capable of mutant rescue, the other truncated proteins are. The conclusion that two TSPt1-like repeats are sufficient for rescuing activity was confirmed by mutant rescue with a mini-transgene.

The lack of gonadal arms in *gon-1 (0)* mutants suggested that the leader cells, which normally govern arm extension, may be defective. To assess whether leader cells were generated during development, we first examined the gonadal cell lineages in *gon-1(0)* mutants during the first two larval stages. Normally, the somatic gonadal progenitor cells, Z1 and Z4, give rise to two leader cells, Z1.aa and Z4.pp, in hermaphrodites, and one leader cell, Z1.pa or Z4.aa, in males (Kimble and Hirsh, 1979). In hermaphrodites, these leader cells are called distal tip cells (DTC), and in males, they are called linker cells (LC). The hermaphrodite distal tip cell is both a leader cell and a regulator of germline proliferation. Kimble, J.E. and J.G. White, "On the control of germ cell development in *Caenorhabditis elegans*, Devel. Biol. 81:208-219 (1981), incorporated herein by reference in its entirety, provides guidance for a skilled artisan on the biology of distal tip cell migration. The information disclosed in that paper can be employed in determining whether an agent modulates cell migration or tissue shaping in a method of the invention.

In *gon-1(0)* hermaphrodites and males, we found that the timing and pattern of cell divisions of Z1 and Z4 and

their descendants were the same as in wild-type during L1 and L2 (data not shown). In particular, Z1.aa and Z1.pp in hermaphrodites and Z1.pa/Z4.aa in males were born at the correct time and place. To ask whether the presumptive hermaphrodite leader cells, Z1.aa and Z4.pp, had adopted the leader fate, we examined expression of a molecular marker for that fate. The *unc-5* gene encodes a netrin receptor and is essential for dorsal migration of leader cells (Leung-Hagesteijn et al, 1992). Using a reporter transgene, *unc-5::lacZ* (J. Culotti, personal communication), we found that *unc-5* expression was the same in wild-type and *gon-1(0)* animals: *unc-5* was not expressed during early larval stages, but was activated in late L3 when the DTCs normally turn dorsally during wild-type gonadogenesis.

Since the hermaphrodite leader cells, Z1.aa and Z4.pp, also control germline proliferation, we next asked if they were correctly specified for that regulatory function. To this end, we examined expression of the *lag-2* gene, which encodes the DTC signal for germline proliferation (Henderson et al., 1994). Using a reporter transgene, *lag-2::*GFP, we found that *lag-2*::GFP expression was similar in wild-type and *gon-1* gonads. Furthermore, we ablated Z1.aa and Z4.pp in *gon-1(0)* mutants and found that germline proliferation was arrested. Therefore, the hermaphrodite DTCs, Z1.aa and Z4.pp, appear to be specified correctly both as leader cells and as regulators of germline proliferation.

Since the leader cells appeared to be specified correctly in *gon-1* mutants, we next examined their ability to migrate and lead arm extension. Normally, the hermaphrodite leader cells (distal tip cells) migrate away from the center of the gonad along the anterior-posterior axis, then reflex dorsally, and migrate back. To compare leader cell migration in wild-type and *gon-1(0)* mutants, we followed their movements throughout gonadal development and at the same time measured gonadal lengths. At the

mid-L1 stage, just prior to division of the leader cell progenitors, Z1 and Z4, the length of the gonad from anterior to posterior end was 19 μm in both wild-type and *gon-1(0)* mutants. Following division of Z1 and Z4 in late L1, a small difference in gonadal length was discerned: 25 μm in wild-type vs. 22 μm in *gon-1* mutants. However, in older larvae with differentiated leader cells, the length differences were dramatic. In *gon-1(0)* hermaphrodites, the distal tip cells had moved little from their birth position and little to no gonad extension had occurred.

A similar defect is observed in males. Normally, the male leader cell (linker cell) migrates anteriorly, then reflexes and migrates to posterior end of the worm. However in *gon-1(0)* males, the linker cell failed to migrate, and little to no extension had occurred. We conclude that *gon-1* is required for leader cell migration and hence gonadal arm extension.

As we observed leader cells during gonadogenesis, we noticed that they assumed an unusual morphology. To explore this further, we examined hermaphrodite DTCs using fluorescence and thin section electron microscopy (EM). Using *lag-2*::GFP, which is expressed in hermaphrodite DTCs and reveals the extent of their cytoplasm (D. Gao and J. Kimble, unpublished), we found that the wild-type and *gon-1(0)* DTCs had dramatically different morphologies. In wild-type, the DTC was crescent-shaped with processes extending around the germ line, while in *gon-1* mutants, it was round and enlarged. Furthermore, the position of the nucleus within the DTC was variable in *gon-1* mutants, whereas in wild-type, it was located at the leading edge of the migrating cell. By EM, we confirmed the difference in morphology between wild-type and *gon-1* leader cells and also discovered a difference in subcellular organization. Whereas wild-type leader cells extend processes along the germline, *gon-1(0)* leader cells do not possess such processes. Furthermore, the plasma membrane is abnormally invaginated in *gon-1(0)* L3 leader cells, and these

membranes accumulate within the cytoplasm of older *gon-1(0)* mutants.

The lack of gonadal arms is not the only defect in *gon-1* mutants. In addition, no gonadal structures (e.g. uterus in hermaphrodites, vas deferens in males) can be discerned. One problem might have been a failure to differentiate gonadal tissues. However, we were able to identify the major somatic gonadal cell types in late L4 *gon-1(0)* mutants. To see somatic gonadal sheath cells, we used *lim*-7::GFP, which expresses Green Fluorescent Protein (GFP) in hermaphrodite sheath cells (O. Hobert, pers. comm.). In wild-type, fluorescence from *lim*-7::GFP encircled the germ cells, while in *gon-1* mutants, only irregularly-shaped patches were observed. Similarly, MH27 antibody, which stains spermathecal cells intensely (den Boer et al., 1998), was present in disorganized patches in *gon-1* mutants. Finally, cells with a typically uterine morphology were present, but no normal uterine structure was found in *gon-1* mutants. Therefore, the gonadal tissues in *gon-1(0)* mutants appear to differentiate correctly.

One simple explanation for the gross morphogenetic defects of mature *gon-1* gonads might have been that all aspects of gonadal morphogenesis are disrupted as a consequence of the defect in leader cell migration. Indeed, by killing the distal tip cells in wild-type animals, we could reproduce the *gon-1* mutant phenotype: arms did not extend and gonadal structures were grossly malformed. However, closer inspection suggests that *gon-1* has a role in gonad morphogenesis independent of leader cells.

To examine the generation of gonadal somatic structures, we removed the germ line (-GL) from *gon-1(0)* to permit formation of an essentially normal somatic gonadal primordium at the early L3 stage and we removed both leader cells (-DTCs) and germline (-GL) from wild-type hermaphrodites as a control. The control animals had no arm extension, but formed a normal somatic gonadal primordium.

A comparison of gonadal structures at the L4 stage, when they are most easily scored, revealed striking differences. While fragments of uterus were present in *gon-1*(-GL) hermaphrodites, no coherent uterus was observed. Furthermore, the *gon-1* (-GL) gonad was small, and most gonadal had extruded from the gonad proper. By contrast, an apparently normal uterus formed in the wild-type animals lacking both DTCs and germ line. Therefore, *gon-1* is required not only for arm extension, but also for morphogenesis of the uterus.

Finally, we asked whether *gon-1* functions in the development of non-gonadal tissues. We assayed embryonic viability, the overall shape of the animal, coordination of its movements, mating behavior in males, the male tail, growth rate, and entry and exit into dauer stage of the life cycle: all were normal in *gon-1(0)* mutants. The normal movement and shape of *gon-1(0)* mutants suggests that *gon-1* is not required generally for cell migration. For example, failure in migration of the CAN neuron causes the tail to wither (Forrester et al., 1998), and defects in axon migration leads to an uncoordinated (Unc) phenotype (Hedgecock et al., 1990). Furthermore, we followed the M sex myoblast and the Q neuroblasts migrations (Antebi et al, 1997)in at least five *gon-1(0)* mutants, and both were normal. We conclude that *gon-1* does not affect cell migrations generally and, furthermore, that *gon-1* does not affect the development of non-gonadal cells, tissues or organs. Finally , we examined the non-gonadal tissues in *gon-1* mutants that had been operated during L1 to remove Z1-Z4, the four gonadal progenitor cells. This experiment was done, because the disorganized gonadal tissues in *gon-1(0)* hermaphrodites often cause the animal to explode during adulthood, preventing examination of their non-gonadal tissues at this stage. Although these gonadless *gon-1* adults had no gross defects, we observed a reproducible vacuolization in the body wall with differential interference contrast microscopy, which was

not seen in similarly treated wild-type animals. However, it must be emphasized that this defect has no apparent developmental consequences. Given the dramatic effects of *gon-1* on gonadogenesis, we suggest that the major role of *gon-1* in development is to control the shape of the gonad.

The wild-type *C. elegans gon-1* sequence is shown in SEQ. ID. NO. 1. The protein encoded by SEQ. ID. NO. 1 is shown in full in SEQ. ID. NO. 2 and in part in comparative Fig. 1C.

PROPHETIC EXAMPLE

A target organism that contains a migration protein is treated with one or more potential modulators of migration of a developing gonadal cell. The organism is preferably a nematode, and is more preferably *C. elegans*. The potential modulating agent is administered in an amount typical of any additive to a culture, preferably at a level of several nanograms to several micrograms per milliliter. The organism can contain a native migration protein or a variant form of a native migration protein, or can express a migration protein from a transgene that can be delivered to the organism in a manner known to those skilled in the art. The protein can also be a chimeric protein expressed from a transgenic polynucleotide that comprises sequences from at least one of the foregoing polynucleotides.

Upon examination, it is observed that one can rescue migration in a target that lacks the migration protein by administering an exogenous polynucleotide that encodes a migration protein. In a target that contains a migration protein, one can also identify administered agents that increase or decrease the migration of a developing gonadal cell. One can also treat the genetic material of the target organism using standard methods and treatments and can then identify genetic changes that increase or decrease migration of developing gonadal cells.

CLAIMS

WE CLAIM:

1. A method for identifying a modulator of a protein that comprises a metalloprotease domain and a thrombospondin domain, the method comprising the steps of:

treating a target organism having a developing gonadal cell responsive to the protein with at least one potential modulator of cell migration; and

observing in the treated target organism a change in migration or shape of the developing gonadal cell attributable to the presence of the at least one modulator.

2. A method as claimed in Claim 1 wherein migration of the developing gonadal cell in the target organism before treatment is absent or reduced relative to a wild type individual.

3. A method as claimed in Claim 1 wherein the treating step restores or enhances migration in the target organism relative to migration before the treating step.

4. A method as claimed in Claim 1 wherein migration of the developing gonadal cell in the target organism before treatment is at a level of a wild type individual.

5. A method as claimed in Claim 1 wherein the treating step reduces migration in the target organism relative to migration before the treating step.

6. A method as claimed in Claim 1 wherein the target organism comprises a protein that comprises a metalloprotease domain and a thrombospondin domain, the protein being selected from the group consisting of a protein encoded by a native polynucleotide coding sequence, a protein encoded by a heterologous polynucleotide coding sequence introduced into the target organism, a protein that shares at least 20% amino acid sequence identity with either of the foregoing and retains an ability to direct cell migration in the target organism, and a chimeric protein encoded at least in part by at least one of the foregoing and introduced into the target organism, the polynucleotide coding sequence being under transcriptional control of a promoter active in a tissue located sufficiently close to the developing gonadal cell so as to signal the cell to migrate.

7. A method as claimed in Claim 6, wherein the native polynucleotide coding sequence is *C. elegans gon-1*.

8. A method as claimed in Claim 6, wherein the heterologous polynucleotide coding sequence is a homolog of *C. elegans gon-1*.

9. A method as claimed in Claim 8 wherein the homolog of *C. elegans gon-1* encodes a metalloprotease enzyme selected from the group consisting of murine ADAMTS-1 protein, bovine procollagen-1 N-proteinase, and human aggrecan-degrading metalloprotease.

10. A method as claimed in Claim 6 wherein the protein is truncated relative to a protein in a wild type individual.

11. A method as claimed in Claim 1 wherein the target organism is a nematode.

12. A method as claimed in Claim 11 wherein the target organism is a nematode selected from the group consisting of *C. elegans* and *C. briggsae*.

13. A method as claimed in Claim 1 wherein the at least one modulator is selected from the group consisting of a nucleic acid molecule, a protein molecule, a sugar, a lipid, an organic molecule, a synthetic or natural pharmaceutical agent, and a mixture thereof.

14. A method for identifying a nucleic acid sequence that affects migration of a developing gonadal cell, the method comprising the steps of:

treating a target organism by a method selected from the group consisting of RNA interference, reverse genetics, and chemical mutagenesis to alter migration or shape of the developing gonadal cell in the treated target organism relative to migration in the target organism before treatment; and

identifying in the treated target organism a nucleic acid sequence affected by the treating step.

15. A method as claimed in Claim 14 wherein the treating step affects a nucleic acid sequence that encodes a protein.

16. A method as claimed in Claim 14 wherein the treating step affects a nucleic acid sequence that regulates nucleic acid transcription or translation.

17. A method as claimed in Claim 14 wherein migration of the developing gonadal cell in the target organism before treatment is absent or reduced relative to a wild type individual.

18. A method as claimed in Claim 14 wherein the treating step restores or enhances migration of the developing gonadal cell in the treated target organism relative to migration before the treating step.

19. A method as claimed in Claim 14 wherein migration of the developing gonadal cell in the target organism before treatment is at a level of a wild type individual.

20. A method as claimed in Claim 14 wherein the treating step reduces migration of the developing gonadal cell in the treated target organism relative to migration before the treating step.

21. A method as claimed in Claim 14, wherein the target organism comprises a protein that directs cell migration, the protein being selected from the group consisting of a protein encoded by a native polynucleotide coding sequence, a protein encoded by a heterologous polynucleotide coding sequence introduced into the target organism, a protein that shares at least 20% amino acid sequence identity with either of the foregoing and retains an ability to direct cell migration in the target organism, and a chimeric protein encoded at least in part by at least one of the foregoing and introduced into the target organism, the polynucleotide coding sequence being under transcriptional control of a promoter active in a tissue located sufficiently close to the developing gonadal cell so as to signal the cell to migrate.

22. A method as claimed in Claim 21 wherein the native polynucleotide coding sequence is *C. elegans gon-1*.

23. A method as claimed in Claim 21 wherein the heterologous polynucleotide coding sequence is a homolog of *C. elegans gon-1*.

24. A method as claimed in Claim 23 wherein the homolog of *C. elegans gon-1* encodes a metalloprotease enzyme selected from the group consisting of murine ADAMTS-1 protein, bovine procollagen-1 N-proteinase, and human aggrecan-degrading metalloprotease.

25. A method as claimed in Claim 21 wherein the protein is truncated relative to a protein in the wild type individual.

26. A method as claimed in Claim 14 wherein the target organism is a nematode.

27. A method as claimed in Claim 26 wherein the target organism is a nematode selected from the group consisting of *C. elegans* and *C. briggsae*.

Fig. 1A

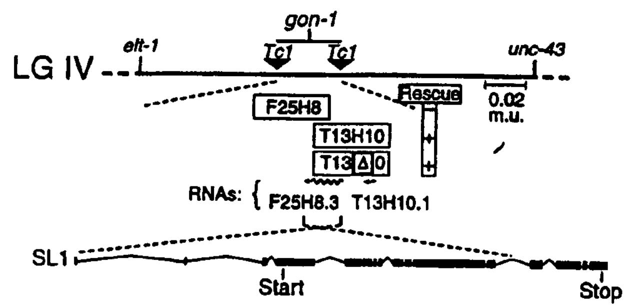
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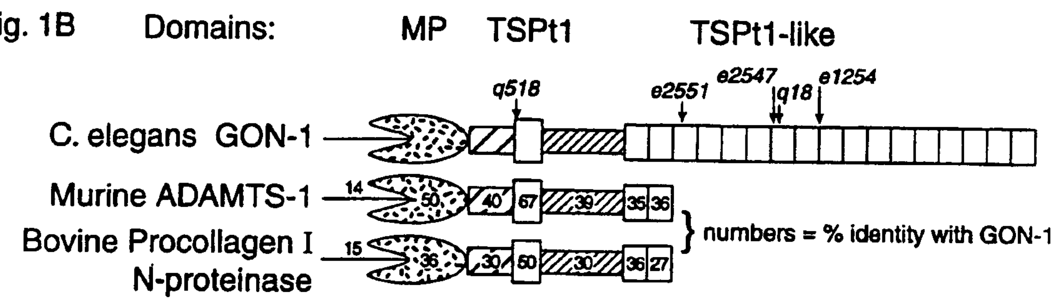
Fig. 1B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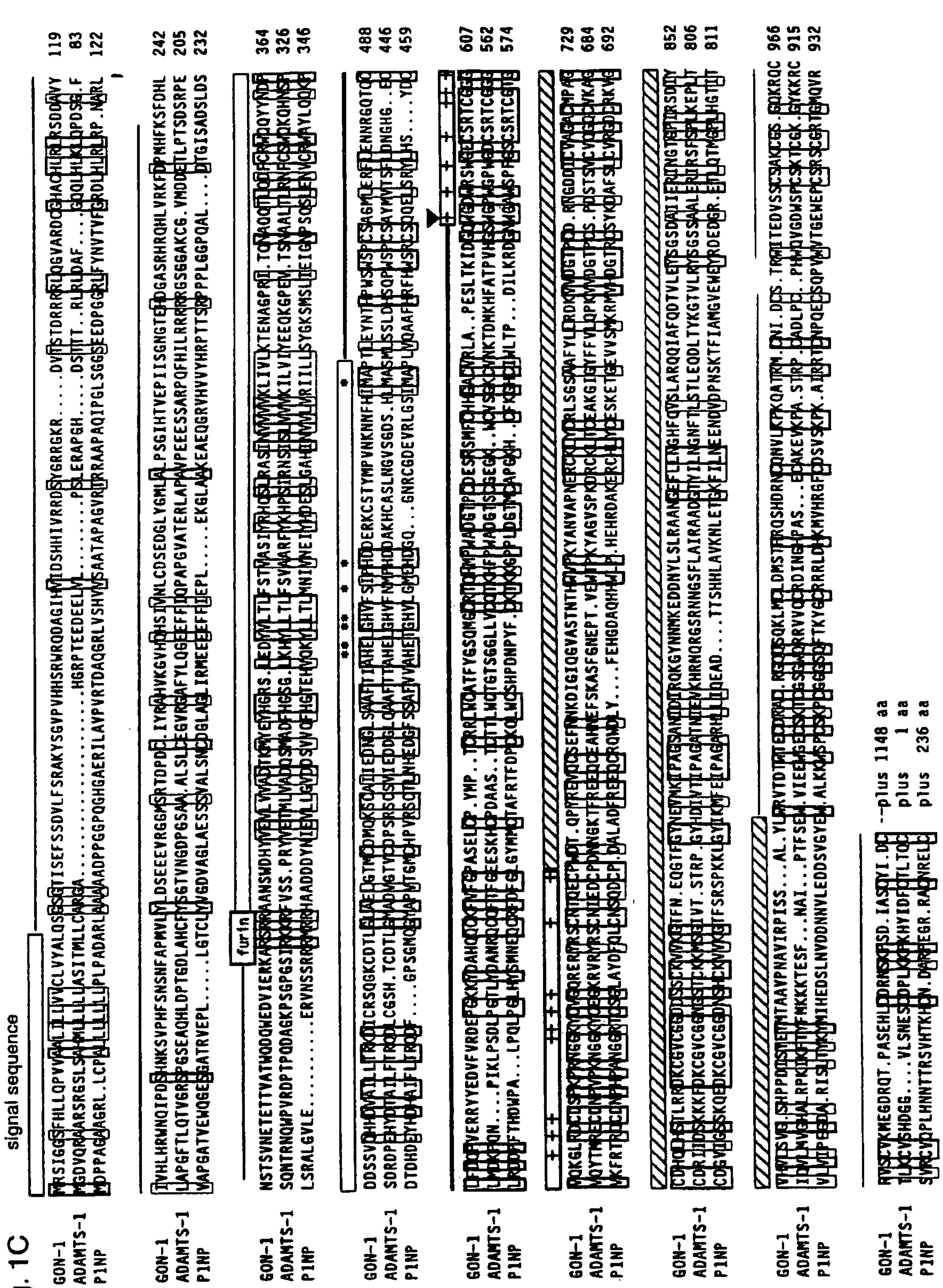

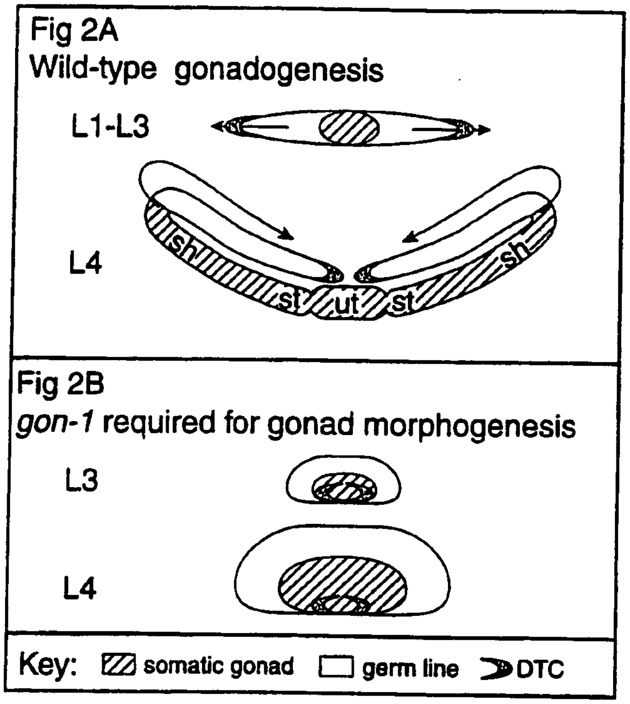
Fig 2A
Wild-type gonadogenesis
L1-L3
L4
sh
st
ut
st
sh
Fig 2B
gon-1 required for gonad morphogenesis
L3
L4
Key:
somatic gonad
germ line
DTC

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Gly Met Ser Arg Thr Asp Pro Asp Cys Ile Tyr Arg Ala His Val Lys
                165                 170                 175

ggt gta cat cag cac agc atc gtc aat tta tgc gac tcg gaa gac gga    576
Gly Val His Gln His Ser Ile Val Asn Leu Cys Asp Ser Glu Asp Gly
            180                 185                 190

ttg tac gga atg ctt gca cta ccc agc gga atc cat acg gtt gag cca    624
Leu Tyr Gly Met Leu Ala Leu Pro Ser Gly Ile His Thr Val Glu Pro
        195                 200                 205

att att agt gga aac gga aca gag cac gac gga gca agt cgc cat agg    672
Ile Ile Ser Gly Asn Gly Thr Glu His Asp Gly Ala Ser Arg His Arg
    210                 215                 220
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caa cat ctc gtc cga aag ttc gat cca atg cac ttc aaa tcg ttt gac 720
Gln His Leu Val Arg Lys Phe Asp Pro Met His Phe Lys Ser Phe Asp
225 230 235 240

cat ctt aac tcg acc agt gtc aac gag acg gag acg acg gtt gcc acg 768
His Leu Asn Ser Thr Ser Val Asn Glu Thr Glu Thr Thr Val Ala Thr
245 250 255

tgg caa gat cag tgg gaa gat gtt att gaa cgc aaa gca aga tcc cga 816
Trp Gln Asp Gln Trp Glu Asp Val Ile Glu Arg Lys Ala Arg Ser Arg
260 265 270

aga gct gcc aac tct tgg gat cac tat gtt gaa gtc ctt gtg gtg gcg 864
Arg Ala Ala Asn Ser Trp Asp His Tyr Val Glu Val Leu Val Val Ala
275 280 285

gat aca aaa atg tac gaa tat cac gga aga tct ctt gaa gac tac gtt 912
Asp Thr Lys Met Tyr Glu Tyr His Gly Arg Ser Leu Glu Asp Tyr Val
290 295 300

ctc act ctc ttc tcc aca gtt gcc tcc atc tat cgt cac caa tcc ctt 960
Leu Thr Leu Phe Ser Thr Val Ala Ser Ile Tyr Arg His Gln Ser Leu
305 310 315 320

cgt gca tct atc aat gtc gtt gtt gtc aag ttg atc gtt ttg aaa acg 1008
Arg Ala Ser Ile Asn Val Val Val Val Lys Leu Ile Val Leu Lys Thr
325 330 335

gaa aac gct gga cca cga atc act cag aac gct caa caa aca ctt caa 1056
Glu Asn Ala Gly Pro Arg Ile Thr Gln Asn Ala Gln Gln Thr Leu Gln
340 345 350

gat ttc tgt aga tgg cag cag tat tac aat gat cca gat gat tcg agt 1104
Asp Phe Cys Arg Trp Gln Gln Tyr Tyr Asn Asp Pro Asp Asp Ser Ser
355 360 365

gtc caa cat cat gac gtt gca atc ctt ttg acg cgt aaa gat att tgt 1152
Val Gln His His Asp Val Ala Ile Leu Leu Thr Arg Lys Asp Ile Cys
370 375 380

cga tca caa gga aaa tgc gat aca ctt gga ctt gct gaa ctt gga aca 1200
Arg Ser Gln Gly Lys Cys Asp Thr Leu Gly Leu Ala Glu Leu Gly Thr
385 390 395 400

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atg tgt gat atg caa aaa agt tgt gca atc ata gaa gac aat gga ttg    1248
Met Cys Asp Met Gln Lys Ser Cys Ala Ile Ile Glu Asp Asn Gly Leu
                405                 410                 415

agt gct gca ttc aca att gct cat gaa ttg ggt cat gtg ttt tcg att    1296
Ser Ala Ala Phe Thr Ile Ala His Glu Leu Gly His Val Phe Ser Ile
            420                 425                 430

cct cat gat gac gaa cga aaa tgc tct acc tac atg ccg gtt aat aag    1344
Pro His Asp Asp Glu Arg Lys Cys Ser Thr Tyr Met Pro Val Asn Lys
        435                 440                 445

aac aac ttc cac ata atg gca cca acg ttg gaa tat aac act cat cca    1392
Asn Asn Phe His Ile Met Ala Pro Thr Leu Glu Tyr Asn Thr His Pro
    450                 455                 460

tgg agt tgg tcg cca tgt tca gct gga atg ctc gaa cga ttc ctc gaa    1440
Trp Ser Trp Ser Pro Cys Ser Ala Gly Met Leu Glu Arg Phe Leu Glu
465                 470                 475                 480

aat aat cga ggt caa act caa tgt cta ttc gat cag ccg gtc gaa cgt    1488
Asn Asn Arg Gly Gln Thr Gln Cys Leu Phe Asp Gln Pro Val Glu Arg
                485                 490                 495

cgt tac tac gag gat gtc ttt gta cgt gat gaa cca gga aag aaa tac    1536
Arg Tyr Tyr Glu Asp Val Phe Val Arg Asp Glu Pro Gly Lys Lys Tyr
            500                 505                 510

gat gct cat caa cag tgc aag ttt gta ttt gga cca gct tct gag ttg    1584
Asp Ala His Gln Gln Cys Lys Phe Val Phe Gly Pro Ala Ser Glu Leu
        515                 520                 525

tgc cct tat atg ccg aca tgc cgc cgt ctt tgg tgt gca aca ttc tac    1632
Cys Pro Tyr Met Pro Thr Cys Arg Arg Leu Trp Cys Ala Thr Phe Tyr
    530                 535                 540

gga agc cag atg ggc tgt cga act cag cat atg cca tgg gcc gac gga    1680
Gly Ser Gln Met Gly Cys Arg Thr Gln His Met Pro Trp Ala Asp Gly
545                 550                 555                 560

act cct tgt gac gaa tca aga agc atg ttc tgt cat cat gga gcc tgt    1728
Thr Pro Cys Asp Glu Ser Arg Ser Met Phe Cys His His Gly Ala Cys
                565                 570                 575
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gtt cgt cta gcc ccc gaa tcc ctt acc aaa att gac gga caa tgg ggt   1776
Val Arg Leu Ala Pro Glu Ser Leu Thr Lys Ile Asp Gly Gln Trp Gly
            580                 585                 590

gac tgg cga tca tgg gga gaa tgc agt cgt act tgt ggt ggt ggt gtt   1824
Asp Trp Arg Ser Trp Gly Glu Cys Ser Arg Thr Cys Gly Gly Gly Val
        595                 600                 605

caa aaa gga tta aga gat tgt gac agc cca aaa cct cga aat ggt gga   1872
Gln Lys Gly Leu Arg Asp Cys Asp Ser Pro Lys Pro Arg Asn Gly Gly
    610                 615                 620

aag tac tgt gtt ggt caa cga gaa cgt tat cgg tca tgt aat aca caa   1920
Lys Tyr Cys Val Gly Gln Arg Glu Arg Tyr Arg Ser Cys Asn Thr Gln
625                 630                 635                 640

gaa tgc cca tgg gat act caa cca tac cgt gaa gtt caa tgt tct gaa   1968
Glu Cys Pro Trp Asp Thr Gln Pro Tyr Arg Glu Val Gln Cys Ser Glu
                645                 650                 655

ttc aac aat aaa gat att gga atc caa ggt gtc gct tca acg aat act   2016
Phe Asn Asn Lys Asp Ile Gly Ile Gln Gly Val Ala Ser Thr Asn Thr
            660                 665                 670

cac tgg gtt cca aaa tat gcg aat gtt gca cca aat gaa cgt tgc aag   2064
His Trp Val Pro Lys Tyr Ala Asn Val Ala Pro Asn Glu Arg Cys Lys
        675                 680                 685

ctg tat tgt cgg ctc agt gga tct gca gcg ttc tat ctg ctt cga gat   2112
Leu Tyr Cys Arg Leu Ser Gly Ser Ala Ala Phe Tyr Leu Leu Arg Asp
    690                 695                 700

aaa gtt gtt gat gga aca cca tgt gat aga aat gga gac gat att tgt   2160
Lys Val Val Asp Gly Thr Pro Cys Asp Arg Asn Gly Asp Asp Ile Cys
705                 710                 715                 720

gta gct gga gct tgt atg cca gca ggc tgt gat cat caa ctt cat tca   2208
Val Ala Gly Ala Cys Met Pro Ala Gly Cys Asp His Gln Leu His Ser
                725                 730                 735

act ctc cga aga gac aaa tgt ggt gtt tgc ggt ggg gat gat tct tcc   2256
Thr Leu Arg Arg Asp Lys Cys Gly Val Cys Gly Gly Asp Asp Ser Ser
            740                 745                 750
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tgt aag gtt gtc aaa gga aca ttt aat gag caa gga acc ttt ggt tat 2304
Cys Lys Val Val Lys Gly Thr Phe Asn Glu Gln Gly Thr Phe Gly Tyr
755 760 765

aac gaa gta atg aag att cca gct ggt tct gca aat att gat atc cgg 2352
Asn Glu Val Met Lys Ile Pro Ala Gly Ser Ala Asn Ile Asp Ile Arg
770 775 780

cag aaa gga tat aat aat atg aaa gaa gat gac aat tat ctt tct ctc 2400
Gln Lys Gly Tyr Asn Asn Met Lys Glu Asp Asp Asn Tyr Leu Ser Leu
785 790 795 800

cgt gcc gcc aat ggt gaa ttc cta ctt aac ggt cat ttc caa gta tca 2448
Arg Ala Ala Asn Gly Glu Phe Leu Leu Asn Gly His Phe Gln Val Ser
805 810 815

ctg gct cgc caa caa att gca ttc caa gac act gtt ctc gaa tat tct 2496
Leu Ala Arg Gln Gln Ile Ala Phe Gln Asp Thr Val Leu Glu Tyr Ser
820 825 830

ggt tct gat gca att att gaa cgg ata aat gga act ggt ccg att aga 2544
Gly Ser Asp Ala Ile Ile Glu Arg Ile Asn Gly Thr Gly Pro Ile Arg
835 840 845

agt gac att tat gtt cat gtt ctt tct gtt ggt agt cat cca ccc gac 2592
Ser Asp Ile Tyr Val His Val Leu Ser Val Gly Ser His Pro Pro Asp
850 855 860

atc tca tat gag tac atg act gcg gct gtt cca aat gct gta att cgg 2640
Ile Ser Tyr Glu Tyr Met Thr Ala Ala Val Pro Asn Ala Val Ile Arg
865 870 875 880

cca ata tcc agt gca ttg tat ttg tgg aga gtt acg gat act tgg aca 2688
Pro Ile Ser Ser Ala Leu Tyr Leu Trp Arg Val Thr Asp Thr Trp Thr
885 890 895

gaa tgt gat aga gcc tgt cgt gga cag caa tcg caa aaa tta atg tgt 2736
Glu Cys Asp Arg Ala Cys Arg Gly Gln Gln Ser Gln Lys Leu Met Cys
900 905 910

ctg gac atg tcg act cat cgt caa agt cat gat aga aat tgt caa aat 2784
Leu Asp Met Ser Thr His Arg Gln Ser His Asp Arg Asn Cys Gln Asn
915 920 925

gtt ctc aaa cca aaa caa gca aca cga atg tgc aat ata gat tgt tct 2832

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Val Leu Lys Pro Lys Gln Ala Thr Arg Met Cys Asn Ile Asp Cys Ser
    930                 935                 940

aca aga tgg atc act gaa gat gtg tct agt tgt agt gcc aaa tgt gga      2880
Thr Arg Trp Ile Thr Glu Asp Val Ser Ser Cys Ser Ala Lys Cys Gly
945                 950                 955                 960

tct gga cag aaa cgt caa cga gtt tct tgc gta aaa atg gag ggt gat      2928
Ser Gly Gln Lys Arg Gln Arg Val Ser Cys Val Lys Met Glu Gly Asp
                965                 970                 975

cgt caa act cca gca tcc gaa cat cta tgt gat cgt aat tca aaa cca      2976
Arg Gln Thr Pro Ala Ser Glu His Leu Cys Asp Arg Asn Ser Lys Pro
            980                 985                 990

tcc gat att gcc agt tgt tac att gac tgc tct gga aga aaa tgg aac      3024
Ser Asp Ile Ala Ser Cys Tyr Ile Asp Cys Ser Gly Arg Lys Trp Asn
        995                 1000                1005

tat gga gaa tgg act tca tgt tct gaa act tgc gga tcg aat gga aaa      3072
Tyr Gly Glu Trp Thr Ser Cys Ser Glu Thr Cys Gly Ser Asn Gly Lys
    1010                1015                1020

atg cat cgg aag tca tat tgc gtt gat gat tcg aat cgt cga gtt gat      3120
Met His Arg Lys Ser Tyr Cys Val Asp Asp Ser Asn Arg Arg Val Asp
1025                1030                1035                1040

gag tca ttg tgc ggc aga gaa cag aaa gag gcg aca gaa cgg gaa tgt      3168
Glu Ser Leu Cys Gly Arg Glu Gln Lys Glu Ala Thr Glu Arg Glu Cys
                1045                1050                1055

aac aga att cca tgt cca aga tgg gtt tat ggg cat tgg tca gag tgc      3216
Asn Arg Ile Pro Cys Pro Arg Trp Val Tyr Gly His Trp Ser Glu Cys
            1060                1065                1070

tct cga agt tgt gat ggt gga gtc aaa atg cgt cat gct caa tgt ttg      3264
Ser Arg Ser Cys Asp Gly Gly Val Lys Met Arg His Ala Gln Cys Leu
        1075                1080                1085

gat gca gcc gat cgg gaa aca cat aca tcc aga tgt ggt cca gca cag      3312
Asp Ala Ala Asp Arg Glu Thr His Thr Ser Arg Cys Gly Pro Ala Gln
    1090                1095                1100
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aca caa gaa cat tgt aat gaa cat gct tgt act tgg tgg cag ttc gga   3360
Thr Gln Glu His Cys Asn Glu His Ala Cys Thr Trp Trp Gln Phe Gly
1105                1110                1115                1120

gtc tgg tct gac tgc tca gct aag tgt gga gat ggt gta cag tat cga   3408
Val Trp Ser Asp Cys Ser Ala Lys Cys Gly Asp Gly Val Gln Tyr Arg
            1125                1130                1135

gac gct aat tgt acc gat cgt cat aga tca gta cta ccg gaa cat cgt   3456
Asp Ala Asn Cys Thr Asp Arg His Arg Ser Val Leu Pro Glu His Arg
        1140                1145                1150

tgc ctt aaa atg gaa aag ata att aca aaa cca tgt cat aga gaa tca   3504
Cys Leu Lys Met Glu Lys Ile Ile Thr Lys Pro Cys His Arg Glu Ser
    1155                1160                1165

tgt cca aaa tat aaa ctt gga gaa tgg tct cag tgt agt gtt tct tgt   3552
Cys Pro Lys Tyr Lys Leu Gly Glu Trp Ser Gln Cys Ser Val Ser Cys
1170                1175                1180

gag gat gga tgg tcg tca aga aga gtt tca tgt gtt tct gga aat gga   3600
Glu Asp Gly Trp Ser Ser Arg Arg Val Ser Cys Val Ser Gly Asn Gly
1185                1190                1195                1200

act gaa gtc gat atg tca ctt tgt ggt act gca tct gat cgg cct gct   3648
Thr Glu Val Asp Met Ser Leu Cys Gly Thr Ala Ser Asp Arg Pro Ala
            1205                1210                1215

tct cat cag aca tgt aat tta ggc act tgc cca ttt tgg aga aat act   3696
Ser His Gln Thr Cys Asn Leu Gly Thr Cys Pro Phe Trp Arg Asn Thr
        1220                1225                1230

gat tgg agt gct tgt tct gta tct tgt gga atc ggt cat cgg gaa cgt   3744
Asp Trp Ser Ala Cys Ser Val Ser Cys Gly Ile Gly His Arg Glu Arg
    1235                1240                1245

aca acc gaa tgc ata tac cgc gaa caa tct gtt gat gct tct ttt tgt   3792
Thr Thr Glu Cys Ile Tyr Arg Glu Gln Ser Val Asp Ala Ser Phe Cys
1250                1255                1260

gga gat acc aaa atg cca gaa act agt caa act tgc cat ctt ctg cca   3840
Gly Asp Thr Lys Met Pro Glu Thr Ser Gln Thr Cys His Leu Leu Pro
1265                1270                1275                1280
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tgt aca tct tgg aaa cca agt cat tgg tcc cct tgc tca gtc act tgt     3888
Cys Thr Ser Trp Lys Pro Ser His Trp Ser Pro Cys Ser Val Thr Cys
                1285                1290                1295

gga tca gga att cag act aga agt gtt tcg tgt act cgt gga tct gaa     3936
Gly Ser Gly Ile Gln Thr Arg Ser Val Ser Cys Thr Arg Gly Ser Glu
            1300                1305                1310

gga act att gtt gat gaa tat ttt tgt gat cga aat act cgt cca cgc     3984
Gly Thr Ile Val Asp Glu Tyr Phe Cys Asp Arg Asn Thr Arg Pro Arg
        1315                1320                1325

cta aaa aag act tgt gaa aaa gat act tgt gat ggg ccc aga gta ctt     4032
Leu Lys Lys Thr Cys Glu Lys Asp Thr Cys Asp Gly Pro Arg Val Leu
    1330                1335                1340

caa aaa ctt caa gcc gac gta cca cca atc cga tgg gca acc gga cca     4080
Gln Lys Leu Gln Ala Asp Val Pro Pro Ile Arg Trp Ala Thr Gly Pro
1345                1350                1355                1360

tgg aca gcc tgt tca gca act tgt ggt aat ggt act caa cgt cgt ctt     4128
Trp Thr Ala Cys Ser Ala Thr Cys Gly Asn Gly Thr Gln Arg Arg Leu
                1365                1370                1375

ctc aag tgc cga gat cat gtt cgt gat ctt cct gat gag tat tgc aat     4176
Leu Lys Cys Arg Asp His Val Arg Asp Leu Pro Asp Glu Tyr Cys Asn
            1380                1385                1390

cat ttg gat aag gaa gta tca aca aga aat tgt cgc ctt cgt gat tgt     4224
His Leu Asp Lys Glu Val Ser Thr Arg Asn Cys Arg Leu Arg Asp Cys
        1395                1400                1405

tca tac tgg aaa atg gcg gaa tgg gaa gag tgt cca gct act tgt gga     4272
Ser Tyr Trp Lys Met Ala Glu Trp Glu Glu Cys Pro Ala Thr Cys Gly
    1410                1415                1420

act cat gtt caa caa agt aga aat gtt aca tgc gtc agt gcg gaa gac     4320
Thr His Val Gln Gln Ser Arg Asn Val Thr Cys Val Ser Ala Glu Asp
1425                1430                1435                1440

ggt ggt cgg acg att ttg aaa gat gtt gat tgt gat gtg caa aag aga     4368
Gly Gly Arg Thr Ile Leu Lys Asp Val Asp Cys Asp Val Gln Lys Arg
                1445                1450                1455
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cca aca agt gca aga aat tgc cga ctt gaa ccc tgt cca aag gga gaa    4416
Pro Thr Ser Ala Arg Asn Cys Arg Leu Glu Pro Cys Pro Lys Gly Glu
            1460                1465                1470

gaa cat att gga tcc tgg att att gga gat tgg tca aaa tgc tct gct    4464
Glu His Ile Gly Ser Trp Ile Ile Gly Asp Trp Ser Lys Cys Ser Ala
        1475                1480                1485

tct tgt ggt ggg gga tgg cgt cgt cgc agt gta tct tgc act tcg tct    4512
Ser Cys Gly Gly Gly Trp Arg Arg Arg Ser Val Ser Cys Thr Ser Ser
    1490                1495                1500

tct tgc gat gaa acc aga aaa cca aag atg ttt gat aaa tgc aat gaa    4560
Ser Cys Asp Glu Thr Arg Lys Pro Lys Met Phe Asp Lys Cys Asn Glu
1505                1510                1515                1520

gaa cta tgt cca cca ctc aca aat aat tct tgg cag ata tct cca tgg    4608
Glu Leu Cys Pro Pro Leu Thr Asn Asn Ser Trp Gln Ile Ser Pro Trp
                1525                1530                1535

act cac tgt tct gta tcg tgt ggc ggg gga gtt caa cgc cgc aaa atc    4656
Thr His Cys Ser Val Ser Cys Gly Gly Gly Val Gln Arg Arg Lys Ile
            1540                1545                1550

tgg tgt gaa gac gtg ctt tcc ggt cgt aaa caa gac gat atc gag tgc    4704
Trp Cys Glu Asp Val Leu Ser Gly Arg Lys Gln Asp Asp Ile Glu Cys
        1555                1560                1565

tca gag att aag cct cgc gaa caa aga gat tgt gaa atg cct cca tgc    4752
Ser Glu Ile Lys Pro Arg Glu Gln Arg Asp Cys Glu Met Pro Pro Cys
    1570                1575                1580

cga tct cat tat cac aac aaa aca tca tca gca tca atg aca tca tta    4800
Arg Ser His Tyr His Asn Lys Thr Ser Ser Ala Ser Met Thr Ser Leu
1585                1590                1595                1600

tca tct tcg aat tca aat acg acg tct tcc gct tcc gct tct tcg ctt    4848
Ser Ser Ser Asn Ser Asn Thr Thr Ser Ser Ala Ser Ala Ser Ser Leu
                1605                1610                1615

cct atc ctt cca ccc gtc gtc tcc tgg caa acg tct gca tgg agc gcg    4896
Pro Ile Leu Pro Pro Val Val Ser Trp Gln Thr Ser Ala Trp Ser Ala
            1620                1625                1630
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tgt tct gca aaa tgc ggt cgt gga acg aaa cga aga gtt gtc gaa tgt     4944
Cys Ser Ala Lys Cys Gly Arg Gly Thr Lys Arg Arg Val Val Glu Cys
            1635                1640                1645

gta aat cca tca tta aat gtg aca gtg gca agt aca gaa tgt gat caa     4992
Val Asn Pro Ser Leu Asn Val Thr Val Ala Ser Thr Glu Cys Asp Gln
    1650                1655                1660

acg aag aaa cca gtt gaa gaa gtt cgt tgt cgt act aaa cat tgc ccg     5040
Thr Lys Lys Pro Val Glu Glu Val Arg Cys Arg Thr Lys His Cys Pro
1665                1670                1675                1680

aga tgg aag act act act tgg agt tcg tgt tct gtc acc tgt ggc aga     5088
Arg Trp Lys Thr Thr Thr Trp Ser Ser Cys Ser Val Thr Cys Gly Arg
                1685                1690                1695

gga atc aga cgt cgt gaa gtt caa tgt tat cgt ggt cgc aag aat ttg     5136
Gly Ile Arg Arg Arg Glu Val Gln Cys Tyr Arg Gly Arg Lys Asn Leu
            1700                1705                1710

gtg tct gat tcg gag tgc aat cca aaa act aag ctc aac tct gtt gcc     5184
Val Ser Asp Ser Glu Cys Asn Pro Lys Thr Lys Leu Asn Ser Val Ala
        1715                1720                1725

aac tgt ttc cca gtg gct tgt cca gct tat aga tgg aat gtt act cca     5232
Asn Cys Phe Pro Val Ala Cys Pro Ala Tyr Arg Trp Asn Val Thr Pro
    1730                1735                1740

tgg agc aag tgc aaa gat gag tgt gct cga gga caa aag caa act cgt     5280
Trp Ser Lys Cys Lys Asp Glu Cys Ala Arg Gly Gln Lys Gln Thr Arg
1745                1750                1755                1760

cgg gtg cac tgt ata agc act tct ggt aaa cga gca gct cca cga atg     5328
Arg Val His Cys Ile Ser Thr Ser Gly Lys Arg Ala Ala Pro Arg Met
                1765                1770                1775

tgt gaa ttg gct cgt gca cca act tcg atc aga gag tgc gat aca tca     5376
Cys Glu Leu Ala Arg Ala Pro Thr Ser Ile Arg Glu Cys Asp Thr Ser
            1780                1785                1790

aat tgt cca tat gag tgg gtg cca gga gat tgg caa acg tgt tca aag     5424
Asn Cys Pro Tyr Glu Trp Val Pro Gly Asp Trp Gln Thr Cys Ser Lys
        1795                1800                1805
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tca tgt gga gaa gga gta cag aca cga gaa gtc aga tgt cgt aga aag     5472
Ser Cys Gly Glu Gly Val Gln Thr Arg Glu Val Arg Cys Arg Arg Lys
   1810                1815                1820

att aat ttt aac tca acc att cca att ata ttt atg ctc gaa gat gaa     5520
Ile Asn Phe Asn Ser Thr Ile Pro Ile Ile Phe Met Leu Glu Asp Glu
1825                1830                1835                1840

cca gct gta cca aaa gag aaa tgt gaa ctt ttc cca aaa cca aat gaa     5568
Pro Ala Val Pro Lys Glu Lys Cys Glu Leu Phe Pro Lys Pro Asn Glu
                1845                1850                1855

tct caa acg tgc gaa ctt aac cca tgc gat tcg gaa ttc aaa tgg agt     5616
Ser Gln Thr Cys Glu Leu Asn Pro Cys Asp Ser Glu Phe Lys Trp Ser
            1860                1865                1870

ttc gga cca tgg ggt gaa tgc tcg aaa aat tgc ggt caa ggt att cga     5664
Phe Gly Pro Trp Gly Glu Cys Ser Lys Asn Cys Gly Gln Gly Ile Arg
        1875                1880                1885

cgt cga cgt gtc aag tgt gtg gcc aat gat ggt cgt cga gtt gaa cga     5712
Arg Arg Arg Val Lys Cys Val Ala Asn Asp Gly Arg Arg Val Glu Arg
   1890                1895                1900

gtc aag tgt acc aca aag aaa cca cgt cga act caa tat tgt ttt gaa     5760
Val Lys Cys Thr Thr Lys Lys Pro Arg Arg Thr Gln Tyr Cys Phe Glu
1905                1910                1915                1920

aga aat tgc ctt ccg tca act tgt cag gag ctt aaa tct cag aat gtt     5808
Arg Asn Cys Leu Pro Ser Thr Cys Gln Glu Leu Lys Ser Gln Asn Val
                1925                1930                1935

aag gct aaa gat gga aat tac act att ctt ctt gac gga ttc act att     5856
Lys Ala Lys Asp Gly Asn Tyr Thr Ile Leu Leu Asp Gly Phe Thr Ile
            1940                1945                1950

gaa att tat tgt cat cga atg aat tca acc att cct aaa gct tat ttg     5904
Glu Ile Tyr Cys His Arg Met Asn Ser Thr Ile Pro Lys Ala Tyr Leu
        1955                1960                1965

aac gtt aat cca aga acc aat ttt gca gag gtt tat gga aaa aaa tta     5952
Asn Val Asn Pro Arg Thr Asn Phe Ala Glu Val Tyr Gly Lys Lys Leu
   1970                1975                1980
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ata tac cct cat act tgc cca ttt aat ggt gat cgt aat gat tca tgc     6000
Ile Tyr Pro His Thr Cys Pro Phe Asn Gly Asp Arg Asn Asp Ser Cys
1985                1990                1995                2000

cat tgt tca gaa gac ggc gat gca agt gct gga ttg acg aga ttc aat     6048
His Cys Ser Glu Asp Gly Asp Ala Ser Ala Gly Leu Thr Arg Phe Asn
                2005                2010                2015

aaa gtt cga ata gat ttg ttg aat aga aag ttc cat ctg gcg gat tat     6096
Lys Val Arg Ile Asp Leu Leu Asn Arg Lys Phe His Leu Ala Asp Tyr
            2020                2025                2030

aca ttt gca aaa cga gaa tat ggt gtt cat gtg cca tat ggt act gcc     6144
Thr Phe Ala Lys Arg Glu Tyr Gly Val His Val Pro Tyr Gly Thr Ala
        2035                2040                2045

ggt gat tgc tac agt atg aaa gat tgt cca cag gga ata ttc tca att     6192
Gly Asp Cys Tyr Ser Met Lys Asp Cys Pro Gln Gly Ile Phe Ser Ile
    2050                2055                2060

gat tta aaa tct gct ggt ctg aaa tta gtt gac gat ctg aat tgg gag     6240
Asp Leu Lys Ser Ala Gly Leu Lys Leu Val Asp Asp Leu Asn Trp Glu
2065                2070                2075                2080

gat caa ggt cat cga aca tcc tct cga atc gat cgt ttt tat aac aat     6288
Asp Gln Gly His Arg Thr Ser Ser Arg Ile Asp Arg Phe Tyr Asn Asn
                2085                2090                2095

gca aaa gtt att ggt cac tgt ggt ggt ttt tgt gga aaa tgc tct cct     6336
Ala Lys Val Ile Gly His Cys Gly Gly Phe Cys Gly Lys Cys Ser Pro
            2100                2105                2110

gag cgg tac aaa gga cta atc ttt gaa gtt aat aca aaa tta tta aat     6384
Glu Arg Tyr Lys Gly Leu Ile Phe Glu Val Asn Thr Lys Leu Leu Asn
        2115                2120                2125

cat gtg aaa aat ggt gga cac att gat gat gaa ttg gat gat gat ggt     6432
His Val Lys Asn Gly Gly His Ile Asp Asp Glu Leu Asp Asp Asp Gly
    2130                2135                2140

ttc tct ggt gac atg gat taa tttttcgat acctaaaagt gtcaaaatct         6483
Phe Ser Gly Asp Met Asp
2145                2150
```

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cgtatgaatc tctacttctc tggtctctta tttcaagttt ttgattcttt tcttttttt 6543

agtttttaat agcattactt cgaatttatt gtcattccct caatcaccta acactaggtt 6603

ttctacatag tatgttcctt gaaaatgttt catgatcaaa ggttacggta cttttg     6659


<210> 2
<211> 2150
<212> PRT
<213> Caenorhabditis elegans

<400> 2
Met Arg Ser Ile Gly Gly Ser Phe His Leu Leu Gln Pro Val Val Ala
1               5                   10                  15

Ala Leu Ile Leu Leu Val Val Cys Leu Val Tyr Ala Leu Gln Ser Gly
            20                  25                  30

Ser Gly Thr Ile Ser Glu Phe Ser Ser Asp Val Leu Phe Ser Arg Ala
        35                  40                  45

Lys Tyr Ser Gly Val Pro Val His His Ser Arg Trp Arg Gln Asp Ala
    50                  55                  60

Gly Ile His Val Ile Asp Ser His His Ile Val Arg Arg Asp Ser Tyr
65                  70                  75                  80

Gly Arg Arg Gly Lys Arg Asp Val Thr Ser Thr Asp Arg Arg Arg Arg
                85                  90                  95

Leu Gln Gly Val Ala Arg Asp Cys Gly His Ala Cys His Leu Arg Leu
            100                 105                 110

Arg Ser Asp Asp Ala Val Tyr Ile Val His Leu His Arg Trp Asn Gln
        115                 120                 125

Ile Pro Asp Ser His Asn Lys Ser Val Pro His Phe Ser Asn Ser Asn
    130                 135                 140

Phe Ala Pro Met Val Leu Tyr Leu Asp Ser Glu Glu Glu Val Arg Gly
145                 150                 155                 160

Gly Met Ser Arg Thr Asp Pro Asp Cys Ile Tyr Arg Ala His Val Lys
                165                 170                 175
```

Gly Val His Gln His Ser Ile Val Asn Leu Cys Asp Ser Glu Asp Gly
180 185 190

Leu Tyr Gly Met Leu Ala Leu Pro Ser Gly Ile His Thr Val Glu Pro
195 200 205

Ile Ile Ser Gly Asn Gly Thr Glu His Asp Gly Ala Ser Arg His Arg
210 215 220

Gln His Leu Val Arg Lys Phe Asp Pro Met His Phe Lys Ser Phe Asp
225 230 235 240

His Leu Asn Ser Thr Ser Val Asn Glu Thr Glu Thr Thr Val Ala Thr
245 250 255

Trp Gln Asp Gln Trp Glu Asp Val Ile Glu Arg Lys Ala Arg Ser Arg
260 265 270

Arg Ala Ala Asn Ser Trp Asp His Tyr Val Glu Val Leu Val Val Ala
275 280 285

Asp Thr Lys Met Tyr Glu Tyr His Gly Arg Ser Leu Glu Asp Tyr Val
290 295 300

Leu Thr Leu Phe Ser Thr Val Ala Ser Ile Tyr Arg His Gln Ser Leu
305 310 315 320

Arg Ala Ser Ile Asn Val Val Val Val Lys Leu Ile Val Leu Lys Thr
325 330 335

Glu Asn Ala Gly Pro Arg Ile Thr Gln Asn Ala Gln Gln Thr Leu Gln
340 345 350

Asp Phe Cys Arg Trp Gln Gln Tyr Tyr Asn Asp Pro Asp Asp Ser Ser
355 360 365

Val Gln His His Asp Val Ala Ile Leu Leu Thr Arg Lys Asp Ile Cys
370 375 380

Arg Ser Gln Gly Lys Cys Asp Thr Leu Gly Leu Ala Glu Leu Gly Thr
385 390 395 400

Met Cys Asp Met Gln Lys Ser Cys Ala Ile Ile Glu Asp Asn Gly Leu
405 410 415

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Ser Ala Ala Phe Thr Ile Ala His Glu Leu Gly His Val Phe Ser Ile
            420                 425                 430

Pro His Asp Asp Glu Arg Lys Cys Ser Thr Tyr Met Pro Val Asn Lys
        435                 440                 445

Asn Asn Phe His Ile Met Ala Pro Thr Leu Glu Tyr Asn Thr His Pro
    450                 455                 460

Trp Ser Trp Ser Pro Cys Ser Ala Gly Met Leu Glu Arg Phe Leu Glu
465                 470                 475                 480

Asn Asn Arg Gly Gln Thr Gln Cys Leu Phe Asp Gln Pro Val Glu Arg
                485                 490                 495

Arg Tyr Tyr Glu Asp Val Phe Val Arg Asp Glu Pro Gly Lys Lys Tyr
            500                 505                 510

Asp Ala His Gln Gln Cys Lys Phe Val Phe Gly Pro Ala Ser Glu Leu
        515                 520                 525

Cys Pro Tyr Met Pro Thr Cys Arg Arg Leu Trp Cys Ala Thr Phe Tyr
    530                 535                 540

Gly Ser Gln Met Gly Cys Arg Thr Gln His Met Pro Trp Ala Asp Gly
545                 550                 555                 560

Thr Pro Cys Asp Glu Ser Arg Ser Met Phe Cys His His Gly Ala Cys
                565                 570                 575

Val Arg Leu Ala Pro Glu Ser Leu Thr Lys Ile Asp Gly Gln Trp Gly
            580                 585                 590

Asp Trp Arg Ser Trp Gly Glu Cys Ser Arg Thr Cys Gly Gly Gly Val
        595                 600                 605

Gln Lys Gly Leu Arg Asp Cys Asp Ser Pro Lys Pro Arg Asn Gly Gly
    610                 615                 620

Lys Tyr Cys Val Gly Gln Arg Glu Arg Tyr Arg Ser Cys Asn Thr Gln
625                 630                 635                 640

Glu Cys Pro Trp Asp Thr Gln Pro Tyr Arg Glu Val Gln Cys Ser Glu
                645                 650                 655
```

Phe Asn Asn Lys Asp Ile Gly Ile Gln Gly Val Ala Ser Thr Asn Thr
660 665 670

His Trp Val Pro Lys Tyr Ala Asn Val Ala Pro Asn Glu Arg Cys Lys
675 680 685

Leu Tyr Cys Arg Leu Ser Gly Ser Ala Ala Phe Tyr Leu Leu Arg Asp
690 695 700

Lys Val Val Asp Gly Thr Pro Cys Asp Arg Asn Gly Asp Asp Ile Cys
705 710 715 720

Val Ala Gly Ala Cys Met Pro Ala Gly Cys Asp His Gln Leu His Ser
725 730 735

Thr Leu Arg Arg Asp Lys Cys Gly Val Cys Gly Gly Asp Asp Ser Ser
740 745 750

Cys Lys Val Val Lys Gly Thr Phe Asn Glu Gln Gly Thr Phe Gly Tyr
755 760 765

Asn Glu Val Met Lys Ile Pro Ala Gly Ser Ala Asn Ile Asp Ile Arg
770 775 780

Gln Lys Gly Tyr Asn Asn Met Lys Glu Asp Asp Asn Tyr Leu Ser Leu
785 790 795 800

Arg Ala Ala Asn Gly Glu Phe Leu Leu Asn Gly His Phe Gln Val Ser
805 810 815

Leu Ala Arg Gln Gln Ile Ala Phe Gln Asp Thr Val Leu Glu Tyr Ser
820 825 830

Gly Ser Asp Ala Ile Ile Glu Arg Ile Asn Gly Thr Gly Pro Ile Arg
835 840 845

Ser Asp Ile Tyr Val His Val Leu Ser Val Gly Ser His Pro Pro Asp
850 855 860

Ile Ser Tyr Glu Tyr Met Thr Ala Ala Val Pro Asn Ala Val Ile Arg
865 870 875 880

Pro Ile Ser Ser Ala Leu Tyr Leu Trp Arg Val Thr Asp Thr Trp Thr
885 890 895

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Glu Cys Asp Arg Ala Cys Arg Gly Gln Gln Ser Gln Lys Leu Met Cys
            900                 905                 910

Leu Asp Met Ser Thr His Arg Gln Ser His Asp Arg Asn Cys Gln Asn
        915                 920                 925

Val Leu Lys Pro Lys Gln Ala Thr Arg Met Cys Asn Ile Asp Cys Ser
    930                 935                 940

Thr Arg Trp Ile Thr Glu Asp Val Ser Ser Cys Ser Ala Lys Cys Gly
945                 950                 955                 960

Ser Gly Gln Lys Arg Gln Arg Val Ser Cys Val Lys Met Glu Gly Asp
                965                 970                 975

Arg Gln Thr Pro Ala Ser Glu His Leu Cys Asp Arg Asn Ser Lys Pro
            980                 985                 990

Ser Asp Ile Ala Ser Cys Tyr Ile Asp Cys Ser Gly Arg Lys Trp Asn
        995                 1000                1005

Tyr Gly Glu Trp Thr Ser Cys Ser Glu Thr Cys Gly Ser Asn Gly Lys
    1010                1015                1020

Met His Arg Lys Ser Tyr Cys Val Asp Asp Ser Asn Arg Arg Val Asp
025                 1030                1035                1040

Glu Ser Leu Cys Gly Arg Glu Gln Lys Glu Ala Thr Glu Arg Glu Cys
                1045                1050                1055

Asn Arg Ile Pro Cys Pro Arg Trp Val Tyr Gly His Trp Ser Glu Cys
            1060                1065                1070

Ser Arg Ser Cys Asp Gly Gly Val Lys Met Arg His Ala Gln Cys Leu
        1075                1080                1085

Asp Ala Ala Asp Arg Glu Thr His Thr Ser Arg Cys Gly Pro Ala Gln
    1090                1095                1100

Thr Gln Glu His Cys Asn Glu His Ala Cys Thr Trp Trp Gln Phe Gly
105                 1110                1115                1120

Val Trp Ser Asp Cys Ser Ala Lys Cys Gly Asp Gly Val Gln Tyr Arg
                1125                1130                1135
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Asp Ala Asn Cys Thr Asp Arg His Arg Ser Val Leu Pro Glu His Arg
            1140                1145                1150

Cys Leu Lys Met Glu Lys Ile Ile Thr Lys Pro Cys His Arg Glu Ser
        1155                1160                1165

Cys Pro Lys Tyr Lys Leu Gly Glu Trp Ser Gln Cys Ser Val Ser Cys
    1170                1175                1180

Glu Asp Gly Trp Ser Ser Arg Arg Val Ser Cys Val Ser Gly Asn Gly
185                 1190                1195                1200

Thr Glu Val Asp Met Ser Leu Cys Gly Thr Ala Ser Asp Arg Pro Ala
                1205                1210                1215

Ser His Gln Thr Cys Asn Leu Gly Thr Cys Pro Phe Trp Arg Asn Thr
            1220                1225                1230

Asp Trp Ser Ala Cys Ser Val Ser Cys Gly Ile Gly His Arg Glu Arg
        1235                1240                1245

Thr Thr Glu Cys Ile Tyr Arg Glu Gln Ser Val Asp Ala Ser Phe Cys
    1250                1255                1260

Gly Asp Thr Lys Met Pro Glu Thr Ser Gln Thr Cys His Leu Leu Pro
265                 1270                1275                1280

Cys Thr Ser Trp Lys Pro Ser His Trp Ser Pro Cys Ser Val Thr Cys
                1285                1290                1295

Gly Ser Gly Ile Gln Thr Arg Ser Val Ser Cys Thr Arg Gly Ser Glu
            1300                1305                1310

Gly Thr Ile Val Asp Glu Tyr Phe Cys Asp Arg Asn Thr Arg Pro Arg
        1315                1320                1325

Leu Lys Lys Thr Cys Glu Lys Asp Thr Cys Asp Gly Pro Arg Val Leu
    1330                1335                1340

Gln Lys Leu Gln Ala Asp Val Pro Pro Ile Arg Trp Ala Thr Gly Pro
1345                1350                1355                1360

Trp Thr Ala Cys Ser Ala Thr Cys Gly Asn Gly Thr Gln Arg Arg Leu
                1365                1370                1375
```

Leu Lys Cys Arg Asp His Val Arg Asp Leu Pro Asp Glu Tyr Cys Asn
1380 1385 1390

His Leu Asp Lys Glu Val Ser Thr Arg Asn Cys Arg Leu Arg Asp Cys
1395 1400 1405

Ser Tyr Trp Lys Met Ala Glu Trp Glu Glu Cys Pro Ala Thr Cys Gly
1410 1415 1420

Thr His Val Gln Gln Ser Arg Asn Val Thr Cys Val Ser Ala Glu Asp
425 1430 1435 1440

Gly Gly Arg Thr Ile Leu Lys Asp Val Asp Cys Asp Val Gln Lys Arg
1445 1450 1455

Pro Thr Ser Ala Arg Asn Cys Arg Leu Glu Pro Cys Pro Lys Gly Glu
1460 1465 1470

Glu His Ile Gly Ser Trp Ile Ile Gly Asp Trp Ser Lys Cys Ser Ala
1475 1480 1485

Ser Cys Gly Gly Gly Trp Arg Arg Arg Ser Val Ser Cys Thr Ser Ser
1490 1495 1500

Ser Cys Asp Glu Thr Arg Lys Pro Lys Met Phe Asp Lys Cys Asn Glu
505 1510 1515 1520

Glu Leu Cys Pro Pro Leu Thr Asn Asn Ser Trp Gln Ile Ser Pro Trp
1525 1530 1535

Thr His Cys Ser Val Ser Cys Gly Gly Gly Val Gln Arg Arg Lys Ile
1540 1545 1550

Trp Cys Glu Asp Val Leu Ser Gly Arg Lys Gln Asp Asp Ile Glu Cys
1555 1560 1565

Ser Glu Ile Lys Pro Arg Glu Gln Arg Asp Cys Glu Met Pro Pro Cys
1570 1575 1580

Arg Ser His Tyr His Asn Lys Thr Ser Ser Ala Ser Met Thr Ser Leu
585 1590 1595 1600

Ser Ser Ser Asn Ser Asn Thr Thr Ser Ser Ala Ser Ala Ser Ser Leu
1605 1610 1615

```
Pro Ile Leu Pro Pro Val Val Ser Trp Gln Thr Ser Ala Trp Ser Ala
            1620                1625                1630

Cys Ser Ala Lys Cys Gly Arg Gly Thr Lys Arg Arg Val Val Glu Cys
        1635                1640                1645

Val Asn Pro Ser Leu Asn Val Thr Val Ala Ser Thr Glu Cys Asp Gln
    1650                1655                1660

Thr Lys Lys Pro Val Glu Glu Val Arg Cys Arg Thr Lys His Cys Pro
665                 1670                1675                1680

Arg Trp Lys Thr Thr Thr Trp Ser Ser Cys Ser Val Thr Cys Gly Arg
                1685                1690                1695

Gly Ile Arg Arg Arg Glu Val Gln Cys Tyr Arg Gly Arg Lys Asn Leu
            1700                1705                1710

Val Ser Asp Ser Glu Cys Asn Pro Lys Thr Lys Leu Asn Ser Val Ala
        1715                1720                1725

Asn Cys Phe Pro Val Ala Cys Pro Ala Tyr Arg Trp Asn Val Thr Pro
    1730                1735                1740

Trp Ser Lys Cys Lys Asp Glu Cys Ala Arg Gly Gln Lys Gln Thr Arg
745                 1750                1755                1760

Arg Val His Cys Ile Ser Thr Ser Gly Lys Arg Ala Ala Pro Arg Met
                1765                1770                1775

Cys Glu Leu Ala Arg Ala Pro Thr Ser Ile Arg Glu Cys Asp Thr Ser
            1780                1785                1790

Asn Cys Pro Tyr Glu Trp Val Pro Gly Asp Trp Gln Thr Cys Ser Lys
        1795                1800                1805

Ser Cys Gly Glu Gly Val Gln Thr Arg Glu Val Arg Cys Arg Arg Lys
    1810                1815                1820

Ile Asn Phe Asn Ser Thr Ile Pro Ile Ile Phe Met Leu Glu Asp Glu
825                 1830                1835                1840

Pro Ala Val Pro Lys Glu Lys Cys Glu Leu Phe Pro Lys Pro Asn Glu
                1845                1850                1855
```

Ser Gln Thr Cys Glu Leu Asn Pro Cys Asp Ser Glu Phe Lys Trp Ser
1860 1865 1870

Phe Gly Pro Trp Gly Glu Cys Ser Lys Asn Cys Gly Gln Gly Ile Arg
1875 1880 1885

Arg Arg Arg Val Lys Cys Val Ala Asn Asp Gly Arg Arg Val Glu Arg
1890 1895 1900

Val Lys Cys Thr Thr Lys Lys Pro Arg Arg Thr Gln Tyr Cys Phe Glu
905 1910 1915 1920

Arg Asn Cys Leu Pro Ser Thr Cys Gln Glu Leu Lys Ser Gln Asn Val
1925 1930 1935

Lys Ala Lys Asp Gly Asn Tyr Thr Ile Leu Leu Asp Gly Phe Thr Ile
1940 1945 1950

Glu Ile Tyr Cys His Arg Met Asn Ser Thr Ile Pro Lys Ala Tyr Leu
1955 1960 1965

Asn Val Asn Pro Arg Thr Asn Phe Ala Glu Val Tyr Gly Lys Lys Leu
1970 1975 1980

Ile Tyr Pro His Thr Cys Pro Phe Asn Gly Asp Arg Asn Asp Ser Cys
985 1990 1995 2000

His Cys Ser Glu Asp Gly Asp Ala Ser Ala Gly Leu Thr Arg Phe Asn
2005 2010 2015

Lys Val Arg Ile Asp Leu Leu Asn Arg Lys Phe His Leu Ala Asp Tyr
2020 2025 2030

Thr Phe Ala Lys Arg Glu Tyr Gly Val His Val Pro Tyr Gly Thr Ala
2035 2040 2045

Gly Asp Cys Tyr Ser Met Lys Asp Cys Pro Gln Gly Ile Phe Ser Ile
2050 2055 2060

Asp Leu Lys Ser Ala Gly Leu Lys Leu Val Asp Asp Leu Asn Trp Glu
065 2070 2075 2080

Asp Gln Gly His Arg Thr Ser Ser Arg Ile Asp Arg Phe Tyr Asn Asn
2085 2090 2095

Ala Lys Val Ile Gly His Cys Gly Gly Phe Cys Gly Lys Cys Ser Pro
2100 2105 2110

Glu Arg Tyr Lys Gly Leu Ile Phe Glu Val Asn Thr Lys Leu Leu Asn
2115 2120 2125

His Val Lys Asn Gly Gly His Ile Asp Asp Glu Leu Asp Asp Asp Gly
2130 2135 2140

Phe Ser Gly Asp Met Asp
145 2150

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